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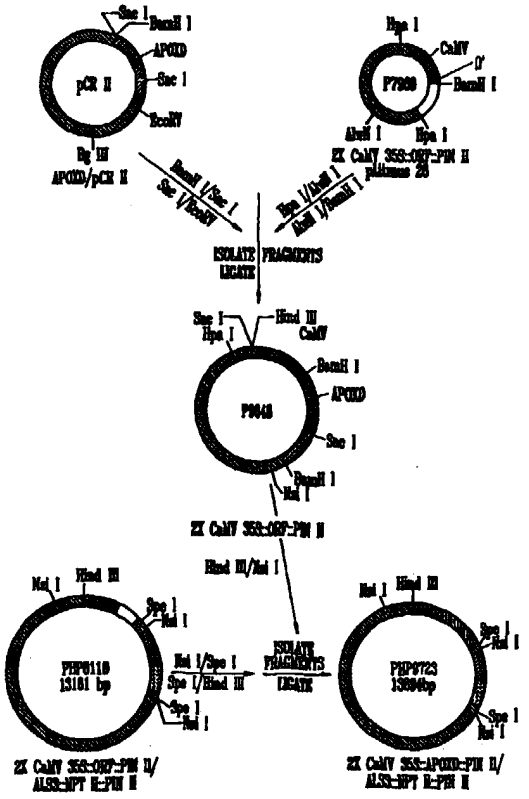
(54) Title: GENE ENCODING OXALATE DECARBOXYLASE FROM ASPERGILLUS PHOENICES

(57) Abstract

A novel nucleic acid sequence encoding *Aspergillus phoenices* oxalate decarboxylase (APOXD) has been determined, as well as the encoded amino acid sequence. The gene and its encoded protein are useful in degrading oxalate, in diagnostic assays of oxalate, and as a selectable marker.



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GENE ENCODING OXALATE DECARBOXYLASE FROM *ASPERGILLUS PHOENICES*

Field of the Invention

This invention relates to a novel nucleic acid sequence encoding oxalate decarboylyase isolated from *Aspergillus phoenices* and to use of the nucleic acid sequence to produce its encoded protein.

Background of the Invention

Oxalic acid (oxalate) is a diffusable toxin associated with various plant diseases, particularly those caused by fungi. Some leafy green vegetables, including spinach and rhubarb, produce oxalate as a nutritional stress factor. When plants containing oxalate are consumed in large amounts, they can be toxic to humans.

Oxalate is used by pathogens to gain access into and subsequently throughout an infected plant. See for example, Mehta and Datta, *The Journal of Biological Chemistry*, 266:23548-23553, 1991; and published PCT Application WO92/14824.

Field crops such as sunflower, bean, canola, alfalfa, soybean, flax, safflower, peanut, clover, as well as numerous vegetable crops, flowers, and trees are susceptible to oxalate-secreting pathogens. For example, fungal species including *Sclerotinia* and *Sclerotium* use oxalic acid to provide an opportunistic route of entry into plants, causing serious damage to crops such as sunflower.

Because of the role of oxalate in plant disease and toxicity, compounds that inhibit oxalate mediated disease, and particularly genes encoding such inhibitory degrading molecules, are greatly needed.

Enzymes that utilize oxalate as a substrate have been identified. These include oxalate oxidase and oxalate decarboxylase. Oxalate oxidase catalyzes the conversion of oxalate to CO_2 and H_2O_2. A gene encoding barley oxalate oxidase has been cloned from a barley root cDNA library and sequenced (See PCT publication No. WO92/14824). A gene encoding wheat oxalate oxidase activity (Germin) has been isolated and sequenced, (PCT publication No. WO 94/13790) and the gene has been introduced into a canola variety. Canola plants harboring the gene appeared to show some

resistance to *Sclerotinia sclerotiorum, in vitro* (Dumas, et al., 1994, Abstracts: *4th Int'l. Congress of Plant Molecular Biology*, #1906).

Oxalate decarboxylase converts oxalate to CO_2 and formic acid. A gene encoding oxalate decarboxylase has been isolated from *Collybia velutipes* (now termed *Flammulina velutipes)* and the cDNA clone has been sequenced (WO94/12622, published 9 June 1994). Oxalate decarboxylase activities have also been described in *Aspergillus niger* and *Aspergillus phoenices* (Emiliani et al., 1964, *ARCH. Biochem. Biophys.* 105:488-493), however the amino acid sequence and nucleic acid sequence encoding these enzyme activities have not been isolated or characterized.

Enzymatic assays for clinical analysis of urinary oxalate provide significant advantages in sensitivity and qualification Obzansky, et al., 1983, *Clinical Chem.* 29:1815-1819. For many reasons, including reactivity with interfering analytes and the high cost of available oxalate oxidase used in this diagnostic assay, alternative enzymes are needed. (Lathika et al., 1995, *Analytical Letters* 28: 425-442).

In this application, we disclose the isolation, cloning, and sequencing of a unique gene encoding an oxalate decarboxylase enzyme from *Aspergillus phoenices*. The gene is useful in producing highly purified *Aspergillus phoenices* oxalate decarboxylase enzyme, in producing transgenic plant cells and plants expressing the enzyme *in vivo*, and in diagnostic assays of oxalate.

Summary of the Invention

The present invention provides a nucleic acid sequence encoding oxalate decarboxylase isolated from *Aspergillus phoenices* (APOXD). The gene sequence [Seq ID No:1], the recombinant protein produced therefrom [Seq ID No:2], and vectors, transformed cells, and plants containing the gene sequence are provided as individual embodiments of the invention, as well as methods using the gene or its encoded protein. The nucleic acid is useful for producing oxalate decarboxylase for commercial applications, including degradation of oxalic acid, protection against oxalic acid toxicity, and diagnostic assays to quantify oxalate.

The nucleic acid of the invention is also useful as a selectable marker. Growth of plant cells in the presence of oxalic acid favors survival of plant cells transformed with the coding sequence of the gene.

The present invention also includes compositions and methods for degrading oxalic acid, in providing protection against oxalic acid toxicity, and in combating and providing protection against plant pathogens that utilize oxalate to gain access to plant tissue or otherwise in the course of the pathogenesis of the disease. Oxalate decarboxylase from *Aspergillis phoenices* (APOXD) of the present invention is combined with an appropriate carrier for delivery to the soil or plants. Alternatively, plant cells are transformed with the nucleic acid sequence of the invention for expression of APOXD *in vivo*.

Brief Description of the Drawings

Figure 1 is a diagram showing a first primer strategy for amplification of a portion of the nucleic acid sequence encoding APOXD.

Figure 2 is a diagram showing the primer position and design of nested, gene-specific primers (arrows above diagram) for 3' RACE and the single gene specific primer (arrow beneath diagram) used for 5' RACE.

Figure 3 is a diagram showing the construction of plasmid pPHP9723 containing the 1.4kb nucleic acid sequence encoding APOXD including leader and pre-sequence.

Figure 4 is a diagram of the plasmid pPHP9723.

Figure 5 is a diagram showing the plasmid pPHP9762 containing the nucleic acid sequence encoding APOXD with the fungal leader and pre-sequence replaced by the plant signal sequence of the wheat oxalate oxidase gene, Germin.

Detailed Description of the Invention

The purified oxalate decarboxylase of the present invention has many commercial uses, including inhibiting oxalate toxicity of plants and preventing pathogenic disease in plants where oxalic acid plays a critical role. It has been suggested that degradation of oxalic acid is a preventative measure, e.g., to prevent invasion of a pathogen into a plant, or during pathogenesis, when oxalic acid concentrations rise (Dumas, et al., 1994, *Supra*). The gene of the invention is also useful as a selectable marker of transformed cells, for diagnostic assay of oxalate, and for production of the enzyme in plants.

Nucleic Acid Sequence Encoding APOXD

A nucleic acid sequence encoding APOXD [Seq. ID No: 1] has now been determined by methods described more fully in the Examples below. Briefly, DNA encoding APOXD was obtained by amplification of genomic *A. phoenices* DNA using a RACE strategy as described in Innis et. al., eds., 1990, *PCR Protocols. A Guide to Methods and Applications*, Academic Press, San Diego, CA, pages 28-38. See also pages 39-45, "Degenerate primers". The nucleic acid sequence and its deduced amino acid sequence [Seq. ID No:2] are shown below in Table 1. The predicted signal peptide [Seq. ID No: 3] and pre-protein [Seq. ID No: 4] are shown along with the potential cleavage site between them as determined by computer analysis using PC gene software (IntelliGenetics, Inc., Mountain View, CA). The mature protein [Seq. ID No: 5] is also indicated. This 1.4 kb sequence encodes a 458 amino acid enzyme subunit with a calculated molecular weight of 51,994 daltons. Southern hybridization indicates that the enzyme is encoded by a single gene in the *Aspergillis phoenices* genome. The plasmid pPHP9685 containing the nucleic acid sequence encoding APOXD as an insert was deposited with the A.T.C.C. on _____, 1997, having Accession No. _____.

TABLE 1

SEQUENCE OF FULL LENGTH APOXD DNA

```
                              Signal Peptide
GGCTTGTCAG GATCCTTCCA AAG ATG CAG CTA ACC CTG CCA CCA CGT CAG CTG  53
                          Met Gln Leu Thr Leu Pro Pro Arg Gln Leu
                           1               5                  10


TTG CTG AGT TTC GCG ACC GTG GCC GCC CTC CTT GAT CCA AGC CAT GGA  101
Leu Leu Ser Phe Ala Thr Val Ala Ala Leu Leu Asp Pro Ser His Gly
               15                  20                  25
Pre-protein
GGC CCG GTC CCT AAC GAA GCG TAC CAG CAA CTA CTG CAG ATT CCC GCC  149
Gly Pro Val Pro Asn Glu Ala Tyr Gln Gln Leu Leu Gln Ile Pro Ala
30                  35                  40
                              Mature Protein
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TCA TCC CCA TCC ATT TTC TTC CAA GAC AAG CCA TTC ACC CCC GAT CAT   197
Ser Ser Pro Ser Ile Phe Phe Gln Asp Lys Pro Phe Thr Pro Asp His
        45                  50                  55

NruI
CGC GAC CCC TAT GAT CAC AAG GTG GAT GCG ATC GGG GAA GGC CAT GAG   245
Arg Asp Pro Tyr Asp His Lys Val Asp Ala Ile Gly Glu Gly His Glu
    60                  65                  70

CCC TTG CCC TGG CGC ATG GGA GAT GGA GCC ACC ATC ATG GGA CCC CGC   293
Pro Leu Pro Trp Arg Met Gly Asp Gly Ala Thr Ile Met Gly Pro Arg
75                  80                  85                  90

AAC AAG GAC CGT GAG CGC CAG AAC CCC GAC ATG CTC CGT CCT CCG AGC   341
Asn Lys Asp Arg Glu Arg Gln Asn Pro Asp Met Leu Arg Pro Pro Ser
                95                  100                 105

ACC GAC CAT GGC AAC ATG CCG AAC ATG CGG TGG AGC TTT GCT GAC TCC   389
Thr Asp His Gly Asn Met Pro Asn Met Arg Trp Ser Phe Ala Asp Ser
            110                 115                 120

CAC ATT CGC ATC GAG GAG GGC GGC TGG ACA CGC CAG ACT ACC GTA CGC   437
His Ile Arg Ile Glu Glu Gly Gly Trp Thr Arg Gln Thr Thr Val Arg
        125                 130                 135

GAG CTG CCA ACG AGC AAG GAG CTT GCG GGT GTA AAC ATG CGC CTC GAT   485
Glu Leu Pro Thr Ser Lys Glu Leu Ala Gly Val Asn Met Arg Leu Asp
    140                 145                 150

GAG GGT GTC ATC CGC GAG TTG CAC TGG CAT CGA GAA GCA GAG TGG GCG   533
Glu Gly Val Ile Arg Glu Leu His Trp His Arg Glu Ala Glu Trp Ala
155                 160                 165                 170

TAT GTG CTG GCC GGA CGT GTA CGA GTG ACT GGC CTT GAC CTG GAG GGA   581
Tyr Val Leu Ala Gly Arg Val Arg Val Thr Gly Leu Asp Leu Glu Gly
                175                 180                 185

GGC AGC TTC ATC GAC GAC CTA GAA GAG GGT GAC CTC TGG TAC TTC CCA   629
Gly Ser Phe Ile Asp Asp Leu Glu Glu Gly Asp Leu Trp Tyr Phe Pro
            190                 195                 200

TCG GGC CAT CCC CAT TCG CTT CAG GGT CTC AGT CCT AAT GGC ACC GAG   677
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Ser Gly His Pro His Ser Leu Gln Gly Leu Ser Pro Asn Gly Thr Glu
        205                 210                 215

TTC TTA CTG ATC TTC GAC GAT GGA AAC TTT TCC GAG GAG TCA ACG TTC   725
Phe Leu Leu Ile Phe Asp Asp Gly Asn Phe Ser Glu Glu Ser Thr Phe
    220                 225                 230

TTG TTG ACC GAC TGG ATC GCA CAT ACA CCC AAG TCT GTC CTC GCC GGA   773
Leu Leu Thr Asp Trp Ile Ala His Thr Pro Lys Ser Val Leu Ala Gly
235                 240                 245                 250

AAC TTC CGC ATG CGC CCA CAA ACA TTT AAG AAC ATC CCA CCA TCT GAA   821
Asn Phe Arg Met Arg Pro Gln Thr Phe Lys Asn Ile Pro Pro Ser Glu
                255                 260                 265

AAG TAC ATC TTC CAG GGC TCT GTC CCA GAC TCT ATT CCC AAA GAG CTC   869
Lys Tyr Ile Phe Gln Gly Ser Val Pro Asp Ser Ile Pro Lys Glu Leu
            270                 275                 280

CCC CGC AAC TTC AAA GCA TCC AAG CAG CGC TTC ACG CAT AAG ATG CTC   917
Pro Arg Asn Phe Lys Ala Ser Lys Gln Arg Phe Thr His Lys Met Leu
        285                 290                 295

GCT CAA AAA CCC GAA CAT ACC TCT GGC GGA GAG GTG CGC ATC ACA GAC   965
Ala Gln Lys Pro Glu His Thr Ser Gly Gly Glu Val Arg Ile Thr Asp
    300                 305                 310

TCG TCC AAC TTT CCC ATC TCC AAG ACG GTC GCG GCC GCC CAC CTG ACC  1013
Ser Ser Asn Phe Pro Ile Ser Lys Thr Val Ala Ala Ala His Leu Thr
315                 320                 325                 330

ATT AAC CCG GGT GCT ATC CGG GAG ATG CAC TGG CAT CCC AAT GCG GAT  1061
Ile Asn Pro Gly Ala Ile Arg Glu Met His Trp His Pro Asn Ala Asp
                335                 340                 345

GAA TGG TCC TAC TTT AAG CGC GGT CGG GCG CGA GTG ACT ATC TTC GCT  1109
Glu Trp Ser Tyr Phe Lys Arg Gly Arg Ala Arg Val Thr Ile Phe Ala
            350                 355                 360

GCT GAA GGT AAT GCT CGT ACG TTC GAC TAC GTA GCG GGA GAT GTG GGC  1157
Ala Glu Gly Asn Ala Arg Thr Phe Asp Tyr Val Ala Gly Asp Val Gly
        365                 370                 375
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ATT GTT CCT CGC AAC ATG GGT CAT TTC ATT GAG AAC CTT AGT GAT GAC  1205
Ile Val Pro Arg Asn Met Gly His Phe Ile Glu Asn Leu Ser Asp Asp
    380                 385                 390

GAG AGG TCG AGG TGT TGG AAA TCT TCC GGG CGG ACC GAT TCC GGG ACT  1253
Glu Arg Ser Arg Cys Trp Lys Ser Ser Gly Arg Thr Asp Ser Gly Thr
395                 400                 405                 410

TTT CTT TGT TCC AGT GGA TGG GAG AGA CGC CGC AGC GGA TGG TGG CAG  1301
Phe Leu Cys Ser Ser Gly Trp Glu Arg Arg Arg Ser Gly Trp Trp Gln
                415                 420                 425

AGC ATG TGT TTA AGG ATG ATC CAG ATG CGG CCA GGG AGT TCC TTA AGA  1349
Ser Met Cys Leu Arg Met Ile Gln Met Arg Pro Gly Ser Ser Leu Arg
            430                 435                 440

GTG TGG AGA GTG GGG AGA AGG ATC CAA TTC GGA GCC CAA GTG AGT AGA  1397
Val Trp Arg Val Gly Arg Arg Ile Gln Phe Gly Ala Gln Val Ser Arg
        445                 450                 455
Stop
TGA GGTTCTACGC GTGTATTTTG CTGATATCAT CGAAGCC                     1437
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APOXD Sequence	Nucleotides	Amino Acids	Seq. ID No.
1.4 kb gene	1-1437		1
Encoded Protein	24-1397	1-458	2
Signal Peptide	24-101	1-26	3
Pre-protein	102-1397	27-458	4
Mature Protein	71-1397	50-458	5

Redundancy in the genetic code permits variation in the gene sequences shown in Table 1. In particular, one skilled in the art will recognize specific codon preferences by a specific host species and can adapt the disclosed sequence as preferred for the desired host. For example, rare codons having a frequency of less than about 20%

in known sequence of the desired host are preferably replaced with higher frequency codons. Codon preferences for a specific organism may be calculated, for example, codon usage tables available on the INTERNET at the following address: http://www.dna.affrc.go.jp/~nakamura/codon.html. One specific program available for Arabidopsis is found at: http://genome-www.stanford.edu/Arabidopsis/codon_usage.html.

Additional sequence modifications are known to enhance protein expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon/intron splice site signals, transposon-like repeats, and other such well-characterized sequences which may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. Where possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures. Other useful modifications include the addition of a translational initiation consensus sequence at the start of the open reading frame, as described in Kozak, 1989, *Mol Cell Biol.* 9:5073-5080.

In addition, the native APOXD gene or a modified version of the APOXD gene might be further optimized for expression by omitting the predicted signal and pre-sequence, replacing the signal sequence with another signal sequence, or replacing the signal and pre-sequence with another signal sequence. Any one of the possible APOXD gene variations may work best when combined with a specific promoter and/or termination sequence.

APOXD Protein

The recombinant APOXD protein produced from the disclosed nucleic acid sequence provides a substantially pure protein useful to degrade oxalate, particularly in applications where highly purified enzymes are required. The recombinant protein may be used in enzymatic assays of oxalate or added to compositions containing oxalate to induce oxalate degradation.

When used externally, the enzyme can be placed in a liquid dispersion or solution, or may be mixed with a carrier solid for application as a dust or powder. The particular method of application and carrier used will be determined by the particular plant and pathogen target. Such methods are known, and are described, for example, in U.S. Patent No. 5,488,035 to Rao.

Gene Delivery

The nucleic acid sequence encoding APOXD may be delivered to plant cells for transient transfections or for incorporation into the plant's genome by methods know in the art. Preferably, the gene is used to stably transform plant cells for expression of the protein *in vivo*.

To accomplish such delivery, the gene containing the coding sequence for APOXD may be attached to regulatory elements needed for the expression of the gene in a particular host cell or system. These regulatory elements include, for example, promoters, terminators, and other elements that permit desired expression of the enzyme in a particular plant host, in a particular tissue or organ of a host such as vascular tissue, root, leaf, or flower, or in response to a particular signal.

Promoters

A promoter is a DNA sequence that directs the transcription of a structural gene, e.g., that portion of the DNA sequence that is transcribed into messenger RNA (mRNA) and then translated into a sequence of amino acids characteristic of a specific polypeptide. Typically, a promoter is located in the 5' region of a gene, proximal to the transcriptional start site. A promoter may be inducible, increasing the rate of transcription in response to an inducing agent. In contrast, a promoter may be constitutive, whereby the rate of transcription is not regulated by an inducing agent. A promoter may be regulated in a tissue-specific or tissue-preferred manner, such that it is only active in transcribing the operably linked coding region in a specific tissue type or types, such as plant leaves, roots, or meristem.

Inducible Promoters

An inducible promoter useful in the present invention is operably linked to a nucleotide sequence encoding APOXD. Optionally, the inducible promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a nucleotide sequence encoding APOXD. With an inducible promoter, the rate of transcription increases in response to an inducing agent.

Any inducible promoter can be used in the present invention to direct transcription of APOXD, including those described in Ward, et al., 1993, *Plant Molecular Biol.* 22: 361:-366. Exemplary inducible promoters include that from the ACE1 system which responds to copper (Mett et al., 1993, *PNAS* 90: 4567-4571); In2 gene promoter

from maize which responds to benzenesulfonamide herbicide safeners (Hershey et al., 1991, *Plant Mol. Biol.* 17:679-690; and the Tet repressor from Tn10 (Hersey, et al., 1991, *Mol. Gen. Genetics* 227:229-237; Gatz, et al., 1994, *Mol.Gen. Genetics* 243:32-38).

A particularly preferred inducible promoter is one that responds to an inducing agent to which plants do not normally respond. One example of such a promoter is the steroid hormone gene promoter. Transcription of the steroid hormone gene promoter is induced by glucocorticosteroid hormone. (Schena et al., 1991, *PNAS U.S.A.* 88:10421)

In the present invention, an expression vector comprises an inducible promoter operably linked to a nucleotide sequence encoding APOXD. The expression vector is introduced into plant cells and presumptively transformed cells are exposed to an inducer of the inducible promoter. The cells are screened for the presence of APOXD proteins by immunoassay methods or by analysis of the enzyme's activity.

Pathogen-Inducible Promoters

A pathogen-inducible promoter of the present invention is an inducible promoter that responds specifically to the inducing agent, oxalic acid, or to plant pathogens such as oxalic acid-producing pathogens including *Sclerotinia sclerotiorum*. Genes that produce transcripts in response to *Sclerotinia* and oxalic acid have been described in Mouley et al., 1992, *Plant Science* 85:51-59. One member of the prp1-1 gene family contains a promoter that is activated in potato during early stages of late blight infection and is described in Martini et al., 1993, *Mol.Gen.Genet.* 236:179-186.

Tissue-specific or Tissue-Preferred Promoters

A tissue specific promoter of the invention is operably linked to a nucleotide sequence encoding APOXD. Optionally, the tissue-specific promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a nucleotide sequence encoding APOXD. Plants transformed with a gene encoding APOXD operably linked to a tissue specific promoter produce APOXD protein exclusively, or preferentially, in a specific tissue.

Any tissue-specific or tissue-preferred promoter can be utilized in the instant invention. Examples of such promoters include a root-preferred promoter such as that from the phaseolin gene as described in Murai et al., 1983, *Science* 222:476-482 and in Sengupta-Gopalan et al., 1985, *PNAS USA* 82:3320-3324; a leaf-specific and light-

induced promoter such as that from *cab* or *rubisco* as described in Simpson et al., 1985, *EMBO J.* 4(11):2723-2729, and in Timko et al., 1985, *Nature* 318:579-582; an anther-specific promoter such as that from *LAT52* as described in Twell et al., 1989, *Mol. Gen. Genet.* 217:240-245; a pollen-specific promoter such as that from *Zm13* as described in Guerrero et al., 1990, *Mol.Gen. Genet.* 224:161-168; and a microspore-preferred promoter such as that from *apg* as described in Twell et al., 1993, *Sex. Plant Reprod.* 6:217-224.

Other tissue-specific promoters useful in the present invention include a phloem-preferred promoter such as that associated with the Arabidopsis sucrose synthase gene as described in Martin et al., 1993, *The Plant Journal* 4(2):367-377; a floral-specific promoter such as that of the Arabidopsis HSP 18.2 gene described in Tsukaya et al., 1993, *Mol.Gen. Genet.* 237:26-32 and of the Arabidopsis HMG2 gene as described in Enjuto et al., 1995, *Plant Cell* 7:517-527.

An expression vector of the present invention comprises a tissue-specific or tissue-preferred promoter operably linked to a nucleotide sequence encoding APOXD. The expression vector is introduced into plant cells. The cells are screened for the presence of APOXD protein by immunological methods or by analysis of enzyme activity.

Constitutive Promoters

A constitutive promoter of the invention is operably linked to a nucleotide sequence encoding APOXD. Optionally, the constitutive promoter is operably linked to a nucleotide sequence encoding a signal sequence which is operably linked to a nucleotide sequence encoding APOXD.

Many different constitutive promoters can be utilized in the instant invention to express APOXD. Examples include promoters from plant viruses such as the 35S promoter from cauliflower mosaic virus (CaMV), as described in Odell et al., 1985, *Nature* 313:810-812, and promoters from genes such as rice actin (McElroy et al., 1990, *Plant Cell* 2:163-171); ubiquitin (Christensen et al., 1989, *Plant Mol. Biol.* 12:619-632; and Christensen et al., 1992, *Plant Mol. Biol* 18:675-689); pEMU (Last et al., 1991, *Theor. Appl. Genet.* 81:581-588); MAS (Velten et al., 1984, *EMBO J.* 3:2723-2730); and maize H3 histone (Lepetit et al., 1992, *Mol.Gen.Genet.* 231:276-285; and Atanassvoa et al., 1992, *Plant Journal* 2(3):291-300).

The ALS promoter, a Xba/*Nco*I fragment 5' to the *Brassica napus* ALS3 structural gene, or a nucleotide sequence having substantial sequence similarity to the XbaI/*Nco*I fragment, represents a particularly useful constitutive promoter, and is described in published PCT Application number WO 96/30530.

In the present invention, an expression vector comprises a constitutive promoter operably linked to a nucleotide sequence encoding APOXD. The expression vector is introduced into plant cells and presumptively transformed cells are screened for the presence of APOXD proteins by immunoassay methods or by analysis of the enzyme's activity.

Additional regulatory elements that may be connected to the APOXD nucleic acid sequence for expression in plant cells include terminators, polyadenylation sequences, and nucleic acid sequences encoding signal peptides that permit localization within a plant cell or secretion of the protein from the cell. Such regulatory elements and methods for adding or exchanging these elements with the regulatory elements of the APOXD gene are known, and include, but are not limited to, 3'termination and/or polyadenylation regions such as those of the *Agrobacterium tumefaciens* nopaline synthase (nos) gene (Bevan et al., 1983, *Nucl. Acids Res.* 11(2):369-385); the potato proteinase inhibitor II (PINII) gene (Keil. et al., 1986, *Nucl. Acids Res.* 14:5641-5650; and An et al., 1989, *Plant Cell* 1:115-122); and the CaMV 19S gene (Mogen et al., 1990, *Plant Cell* 2:1261-1272).

Plant signal sequences, including, but not limited to, signal-peptide encoding DNA/RNA sequences which target proteins to the extracellular matrix of the plant cell (Dratewka-Kos, et al., *J. Biol. Chem.* 264:4896-4900, 1989) and the Nicotiana plumbaginifolia extensin gene (DeLoose, et al., *Gene* 99:95-100, 1991), or signal peptides which target proteins to the vacuole like the sweet potato sporamin gene (Matsuoka, et al., *PNAS* 88:834, 1991) and the barley lectin gene (Wilkins, et al., *Plant Cell*, 2:301-313, 1990), or signals which cause proteins to be secreted such as that of PRIb (Lund, et al., *Plant Mol. Biol.* 18:47-53, 1992), or those which target proteins to the plastids such as that of rapeseed enoyl-Acp reductase (Verwoert, et al., *Plant Mol. Biol.* 26:189-202, 1994) are useful in the invention.

Gene Transformation Methods

Numerous methods for introducing foreign genes into plants are known and can be used to insert the APOXD gene into a plant host, including biological and physical plant transformation protocols. See, for example, Miki et al., 1993, "Procedure for Introducing Foreign DNA into Plants" in: *Methods in Plant Molecular Biology and Biotechnology*, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pages 67-88. The methods chosen vary with the host plant, and include chemical transfection methods such as calcium phosphate, microorganism-mediated gene transfer such as *Agrobacterium* (Horsch, et al., *Science* 227:1229-31, 1985), electroporation, micro-injection, and biolistic bombardment.

Expression cassettes and vectors and *in vitro* culture methods for plant cell or tissue transformation and regeneration of plants are known and available. See, for example, Gruber, et al., 1993, "Vectors for Plant Transformation" In: *Methods in Plant Molecular Biology and Biotechnology*, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pages 89-119.

***Agrobacterium*-mediated Transformation**

The most widely utilized method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. *A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectfully, carry genes responsible for genetic transformation of plants. See, for example, Kado, 1991, *Crit. Rev.Plant Sci.* 10(1):1-32. Descriptions of the *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided in Gruber et al., *supra;* Miki, et al., *supra*; and Moloney, et al., 1989, *Plant Cell Reports* 8:238.

Direct Gene Transfer

Despite the fact that the host range for *Agrobacterium*-mediated transformation is broad, some major cereal crop species and gymnosperms have generally be recalcitrant to this mode of gene transfer, even though some success has recently been achieved in rice (Hiei et al., 1994, *The Plant Journal* 6(2):271-282). Several methods of plant transformation, collectively referred to as direct gene transfer, have been developed as an alternative to *Agrobacterium*-mediated transformation.

A generally applicable method of plant transformation is microprojectile-mediated transformation, where DNA is carried on the surface of microprojectiles measuring about 1 to 4 Tm. The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate the plant cell walls and membranes. (Sanford et al., 1987, *Part.Sci. Technol* 5:27; Sanford, 1988, *Trends Biotech* 6:299; Sanford, 1990, *Physiol.* Plant 79:206; Klein et al., 1992, *Biotechnology* 10:268)

Another method for physical delivery of DNA to plants is sonication of target cells as described in Zhang et al., 1991, *Bio/Technology* 9:996. Alternatively, liposome or spheroplast fusions have been used to introduce expression vectors into plants. See, for example, Deshayes et al., 1985, *EMBO J.* 4:2731-2737; and Christou, et al., 1987, *PNAS USA* 84:3962-3966. Direct uptake of DNA into protoplasts using $CaCl_2$ precipitation, polyvinyl alcohol or poly-L-ornithine have also been reported. See, for example, Hain et al., 1985, *Mol. Gen.Genet.* 199:161; and Draper, et al., 1982, *Plant & Cell Physiol.* 23:451.

Electroporation of protoplasts and whole cells and tissues has also been described. See, for example, D'Halluin, et al., 1992, *Plant Cell* 4:1495-1505; and Spencer, et al., 1994, *Plant Mol.Biol.* 24:51-61.

Particle Wounding/*Agrobacterium* Delivery

Another useful basic transformation protocol involves a combination of wounding by particle bombardment, followed by use of *Agrobacterium* for DNA delivery, as described by Bidney, et al. 1992, *Plant Mol. Biol.* 18:301-313. Useful plasmids for plant transformation include pPHP9762 shown in Figure 5. The binary backbone for pPHP9762 is pPHP6333. See Bevan, 1984, *Nucleic Acids Research* 12:8711-8721. This protocol is preferred for transformation of sunflower plants, and employs either the "intact meristem" method or the "split meristem" method.

In general, the intact meristem transformation method (Bidney, et al., *Supra*) involves imbibing seed for 24 hours in the dark, removing the cotyledons and root radical, followed by culturing of the meristem explants. Twenty-four hours later, the primary leaves are removed to expose the apical meristem. The explants are placed apical dome side up and bombarded, e.g., twice with particles, followed by co-cultivation with *Agrobacterium*. To start the co-cultivation for intact meristems, *Agrobacterium* is placed

on the meristem. After about a 3-day co-cultivation period the meristems are transferred to culture medium with cefotaxime (plus kanamycin for the NPTII selection). Selection can also be done using kanamycin.

The split meristem method involves imbibing seed, breaking of the cotyledons to produce a clean fracture at the plane of the embryonic axis, excising the root tip and then bisecting the explants longitudinally between the primordial leaves (Malone-Schoneberg et al., 1994, *Plant Science* 103:199-207). The two halves are placed cut surface up on the medium then bombarded twice with particles, followed by co-cultivation with *Agrobacterium*. For split meristems, after bombardment the meristems are placed in an *Agrobacterium* suspension for 30 minutes. They are then removed from the suspension onto solid culture medium for three day co-cultivation. After this period, the meristems are transferred to fresh medium with cefotaxime (plus kanamycin for selection).

Transfer by Plant Breeding

Alternatively, once a single transformed plant has been obtained by the foregoing recombinant DNA method, conventional plant breeding methods can be used to transfer the structural gene and associated regulatory sequences via crossing and backcrossing. Such intermediate methods will comprise the further steps of: (1) sexually crossing the disease-resistant plant with a plant from the disease-susceptible taxon; (2) recovering reproductive material from the progeny of the cross; and (3) growing disease-resistant plants from the reproductive material. Where desirable or necessary, the agronomic characteristics of the susceptible taxon can be substantially preserved by expanding this method to include the further steps of repetitively: (1) backcrossing the disease-resistant progeny with disease-susceptible plants from the susceptible taxon; and (2) selecting for expression of APOXD activity (or an associated marker gene) among the progeny of the backcross, until the desired percentage of the characteristics of the susceptible taxon are present in the progeny along with the gene imparting APOXD activity.

By the term "taxon" herein is meant a unit of botanical classification of genus or lower. It thus includes genus, species, cultivars, varieties, variants and other minor taxonomic groups which lack a consistent nomenclature.

Assay Methods

Transgenic plant cells, callus, tissues, shoots, and transgenic plants are tested for the presence of the APOXD gene by DNA analysis (Southern blot or PCR) and for expression of the gene by immunoassay or by assay of oxalate decarboxylase activity. Tolerance to exogenous oxalic acid can also be used as a functional test of enzyme expression in transformed plants.

APOXD ELISA

Transgenic cells, callus, plants and the like are screened for the expression of APOXD protein by immunological assays, including ELISA. Anti-APOXD antibodies are generated against APOXD preparations by known methods and are used in typical ELISA reactions. Polyclonal anti-APOXD can, for example, detect a range of about 10-100 pg APOXD protein in transgenic plant tissues.

In a suitable method for an APOXD-ELISA assay, fresh leaf or callus tissue is homogenized and centrifuged. An aliquot of the supernatant is added to a microtiter plate with a first anti-APOXD antibody and incubated for sufficient time for antibody-antigen reaction. The bound antibody is then reacted with a second antibody linked to a marker, which marker is developed or otherwise converted to a detectable signal correlated to the amount of APOXD protein in the sample. Any of the known methods for producing antibodies and utilizing such antibodies in an immunoassay can be used to determine the amount of APOXD expressed in transgenic plant cells and tissues of the invention.

Oxalate Decarboxylase Assay

Transgenic cells, tissue, or plants expressing the APOXD gene are assayed for enzyme activity to verify expression of the gene. In general, the cells or tissue is frozen in liquid nitrogen, placed on a lyophilizer overnight to dehydrate, then crushed into a fine powder for use in the assay reaction. Leaf tissue is homogenized as fresh tissue in the reaction mixture, or dehydrated and treated as described above.

A typical assay reaction is begun by adding 0.75 mg of powdered tissue, such as callus, to 1 ml of oxalate decarboxylase reaction mixture: 900 Tl 0.2 M sodium phosphate buffer, pH 5.0, and 100 Tl of 10 mM sodium oxalate, pH 5.0. The reaction is incubated at room temperature for 3 hours with gentle mixing, and is stopped by the addition of 150 Tl of 1 M Tris-HCl, pH 7.0. The mixture is centrifuged, and an aliquot is

placed in a cuvette with NAD (600 Tg) and formate dehydrogenase (200 Tg). The absorbance at 340 nm is correlated to the activity of the APOXD enzyme.

Use of Oxalate Decarboxylase as a Selectable Marker

Oxalate decarboxylase is useful in selecting successful transformants, e.g., as a selectable marker. Growth of plant cells in the presence of oxalic acid favors the survival of plant cells that have been transformed with a gene encoding an oxalate-degrading enzyme, such as APOXD. In published PCT application WO 94/13790, herein incorporated by reference, plant cells grown on a selection medium containing oxalic acid (and all of the elements necessary for multiplication and differentiation of plant cells) demonstrated selection of only those cells transformed with and expressing oxalate oxidase. In like manner, transformation and expression of the gene encoding APOXD in plant cells is used to degrade oxalic acid present in the media and allow the growth of only APOXD-gene transformed cells.

Production of APOXD in Plants

Trangenic plants of the present invention, expressing the APOXD gene, are used to produce oxalate decarboxylase in commercial quantities. The gene transformation and assay selection techniques described above yield a plurality of transgenic plants which are grown and harvested in a conventional manner. Oxalate decarboxylase is extracted from the plant tissue or from total plant biomass. Oxalate decarboxylase extraction from biomass is accomplished by known methods. See for example, Heney and Orr, 1981, *Anal. Biochem.* 114:92-96.

In any extraction methodology, losses of material are expected and costs of the procedure are also considered. Accordingly, a minimum level of expression of oxalate decarboxylase is required for the process to be deemed economically worthwhile. The terms "commercial" and "commercial quantities" here denote a level of expression where at least 0.1% of the total extracted protein is oxalate decarboxylase. Higher levels of oxalate decarboxylase expression are preferred.

Diagnostic Oxalate Assay

Clinical measurement of oxalic acid in urine is important, for example, in the diagnosis and treatment of patients with urinary tract disorders or hyperoxaluric syndromes. The recombinant APOXD enzyme of the invention is preferably immobilized onto beads or solid support, or added in aqueous solution to a sample for quantitation of

oxalate. As discussed above, oxalate decarboxylase catalyzes the conversion of oxalate to CO_2 and formic acid. A variety of detection systems can be utilized to quantify this enzyme catalyzed conversion, including methods for detecting an increase in CO_2, or for detecting an increase in formic acid.

For example, the conversion of oxalate to formic acid and CO_2 is assayed by determining formate production via the reduction of NAD in the presence of formate dehydrogenase. This method is described in Lung, et al., 1994, *J. Bacteriology*, 176:2468-2472 and Johnson, et al., 1964, *Biochem. Biophys. Acta* 89:35.

A calibration curve is generated using known amounts of oxalic acid. The amount of oxalate in a specimen is extrapolated from the standard curve.

Other enzymatic assays and the like are adapted by known methods to utilize the APOXD enzyme to detect conversion of oxalate.

EXAMPLES

The invention is described more fully below in the following Examples, which are exemplary in nature and are not intended to limit the scope of the invention in any way.

Example 1

Cloning of the Gene Encoding APOXD

Protein Sequence

A commercial preparation of *A. phoenices* oxalate decarboxylase enzyme was obtained from Boehringer Mannheim. (Catalog #479 586) SDS polyacrylamide gel electrophoresis was used to determine the purity of the enzyme. Only one dark band appeared following Coomassie blue staining of the polyacrylamide gel (12.5%). This band was about 49 kd in size, as determined by comparison to molecular weight markers. Aliquots of the preparation were sent to the University of Michigan for sequence analysis by Edman degradation on an automated protein sequencer. Preparative polyacrylamide gels were run and the APOXD band was isolated from the gel prior to sequencing. The protein was first sequenced at the amino terminus. Proteins were chemically cleaved into fragments by cyanogen bromide, size separated on polyacrylamide gels, and isolated as bands on the gel for further preparation and sequencing. The results of the sequencing are shown below in Table 2.

TABLE 2

Peptide	Sequence*	Seq. ID No.
amino terminus	Gln Asp Lys Pro Phe Thr Pro Asp His Arg Asp Pro Tyr Asp His Lys Val Asp Ala Ile Gly Glu X His Glu Pro Leu	6
fragment 1	Val Ile Arg Glu Leu His Trp His Arg Glu Ala Gly	7
fragment 2	Arg Leu Asp Glu Gly Val Ile Arg Glu Leu His Cys His Arg Glu Ala Glu	8
fragment 3	Ser Tyr Phe Lys Arg Gly Arg Ala Arg Tyr Thr Ile Phe Ala Ala Glu Gly Asn Ala Arg	9
fragment 4	Ser Ala His Thr Pro Pro Ser Val Leu Ala Gly Asn	10

* X = Unknown.

PCR Amplification of Genomic *A. phoenices*

Genomic DNA was used as the PCR template to amplify the APOXD sequence. *Aspergillus phoenices* was obtained from the American Type Culture Collection (ATCC), Rockville, MD. Cultures were established on solid potato dextrose agar medium (Difco formulation). Liquid stationary cultures were started from culture plates by innoculatory spores in a minimal growth medium previously described for the culture of *Aspergillus* strains (Emiliani, et al., 1964, *Arch. Biochem. Biophys* 105:488-493, cited above).

To isolate DNA, mycelial mats were recovered from 4-day liquid stationary cultures, washed in cold water, and blotted dry. The tissue was then frozen in liquid nitrogen, ground by mortar and pestle, and stored frozen at -80°C. DNA was extracted by the method described for fungal mycelium in Sunis et al. (eds.), 1990, *PCR protocols*, pages 282-287.

PCR Strategy

As diagrammed in Figure 1, primers were designed for both the N-terminal protein sequence and for an internal peptide fragment. One set of primers (PHN 11337 [Seq ID No. 11] and PHN 11339 [Seq ID No. 12]) was designed with nearly full degeneracy. A second set of primers (PHN 11471 [Seq. ID No. 13] and PHN 11476 [Seq ID No. 14]) was designed with no degeneracy. These were based on a codon usage table for *Aspergillus niger* generated using the Wisconsin Sequence Analysis Package (GCG)

(Genetics Computer Group, Inc., Madison, WI). The sequences of these primers is shown in Table 3, below, and diagrammatically in Figure 1. Table 3 shows the degenerate primer mixtures using IUPAC designations, as described in Cornish-Bowden, 1985, *Nucleic Acids Res.* 13:3021-3030. The IUPAC nucleic acid symbols include: Y=C or T; N=A, T, C, or G; R=A or G; D=A, T, or G; and V=A, C, or G. Both of these PCR strategies were successful in amplifying a DNA fragment, shown in Table 4, having homology to the protein sequence data shown in Table 2.

TABLE 3

Primer Sets (5'-3')		Seq. ID #
CAU CAU CAU CAU CCA TGG GAY CAY CGN GAY CCY TA	PHN11337	11
CUA CUA CUA CUA AGG CCT GTG NRR YTC NCG DAT VA	PHN11339	12
CA CCA TGG TAC GAT CAC AAG GT	PHN11471	13
TCA GGC CTT GCC AGT GCA ACT	PHN11476	14

PCR reactions were set up containing increasing quantities of *A. phoenices* genomic DNA, in the range of 1-10 nanograms, and various oligonucleotide primer sets. Degenerate primers were added at a ten-fold higher concentration than that standardly used, due to their degeneracy. All other conditions for PCR were standard, essentially as described in Innis, et al., 1990, *PCR Protocols*, pages 282-287, except for the annealing temperatures for the primers. These temperatures were determined on an individual basis using the Oligo 4.0 computer program for analysis as described in Rychlik et al., 1989, *Nuc.Acids Res.* 17:8543-8551. Specifically, the primers and annealing temperatures were :

primer	first 5 cycles	next 30 cycles
PHN 11337	54° C	60° C
PHN 11339	54° C	60° C
PHN 11471	50° C	58° C
PHN 11476	50° C	58° C

Transformation and Sequencing

PCR amplification products were ligated into pCR II using the TA Cloning Kit (InVitrogen, San Diego, CA), and transformed into *E.coli* strain DH5α competent cells

(Life Technologies, Gathersburg, MD) according to the protocol provided with the strain, for cloning and sequencing. Transformed bacteria with plasmid insertions were selected on medium 34Z (LB agar plates containing 100 mg/l carbenicillin) using standard X-GAL selection protocols (Ausubel, et al., eds, 1989, *Current Protocols in Molecular Biology*, pages 1.0.3-1.15.8). Briefly, white colonies were picked with an inoculating loop and inoculated directly into a PCR reaction mixture containing primers specific to the universal and reverse promoter regions just outside the multiple cloning site. The remaining innoculum on the loop was used to streak a plate of 34Z medium and numbered to correspond to the PCR reaction. Successful amplification of an inserted PCR fragment resulted in a band on an ethidium bromide stained agaraose gel which was slightly larger than the size of the insert. Bacterial isolates with an insert of the correct size were inoculated into shaking liquid cultures and subsequently used for plasmid isolation protocols, followed by sequencing of the insert of interest.

Sequence quality plasmid was prepared by using the Nucleobond P-100 plasmid isolation kit (Machery-Nagle GmBH & Co., Cat.No. BP 101352m distributed by the Nest Group, Southboro, MA). This kit uses an alkaline lysis step and is followed by an ion exchange silica column purification step. Plasmid and gene specific primers were sent to Iowa State University to be sequenced on an automated, ABI DNA Sequencing machine.

The degenerate primer PCR experiment resulted in the amplification of a 0.4 kb band, which was sequenced and determined to have a deduced amino acid sequence matching the protein data in Table 2. The non-degenerate primer experiment resulted in DNA fragments of various sizes. One fragment was about 0.4 kb in length and encoded a protein having homology to the protein sequence data of Table 2. The region of the APOXD gene that was amplified by both primer sets was nearly the same, so DNA sequence data for the amplified fragments was compiled, and the sequence of the compiled APOXD genomic fragment is shown in Table 4 [Seq ID No. 15] together with its deduced amino acid sequence [Seq ID Nos. 16 and 29]. The underlined amino acid sequences were represented in the original protein sequence analysis data (Table 2).

TABLE 4
APOXD FRAGMENT

```
          10          20          30          40
ACG ATC ACA AGG TGG ATG CGA TCG GGG AAG GCC ATG AGC CCT TGC CCT
    Asp His Lys Val Asp Ala Ile Gly Glu Gly His Glu Pro Leu Pro

50          60          70          80          90
GGC GCA TGG GAG ATG GAG CCA CCA TCA TGG GAC CCC GCA ACA AGG ACC
Trp Arg Met Gly Asp Gly Ala Thr Ile Met Gly Pro Arg Asn Lys Asp

    100         110         120         130         140
GTG AGC GCC AGA ACC CCG ACA TGC TCC GTC CTC CGA GCA CCG ACC ATG
Arg Glu Arg Gln Asn Pro Asp Met Leu Arg Pro Pro Ser Thr Asp His

    150         160         170         180         190
GCA ACA TGC CGA ACA TGC GGT GGA GCT TTG CTG ACT CCC ACA TTC GCA
Gly Asn Met Pro Asn Met Arg Trp Ser Phe Ala Asp Ser His Ile Arg

       200         210         220         230         240
TCG AGG TAA GCC CTT CGA GGG TTT TGT GTA CGA CAA GCA AAA TAG GCT
Ile Glu

          250         260         270         280
AAT GCA CTG CAG GAG GGC GGC TGG ACA CGC CAG ACT ACC GTA CGC GAG
                      Gly Trp Thr Arg Gln Thr Thr Val Arg Glu

 290         300         310         320         330
CTG CCA ACG AGC AAG GAG CTT GCG GGT GTA AAC ATG CGC CTC GAT GAG
Leu Pro Thr Ser Lys Glu Leu Ala Gly Val Asn Met Arg Leu Asp Glu

    340         350         360         370         380
GGT GTC ATC CGC GAG TTG CAC TGG CAA GGG CTG AAG GCG AAT TCC AGC
Gly Val Ile Arg Glu Leu His Trp

    390         400         410         420         430
ACA CTG GCG GCC GTT ACT AGT GGA TCC GAG CTC GGT ACC AAG CTT GAT

GC ATAGCT
```

3' RACE

Nested oligonucleotide primers were designed based on the genomic DNA sequence fragment which was previously amplified (Table 4) and used for 3' RACE to enhance gene specific amplification. The nested primer design is diagrammatically shown in Figure 2 and the nucleic acid sequences of the primers is shown below in Table 5. Arrows represent the gene specific primers (from top to bottom) PHN 11811, PHN 11810, and the oligo dT based 3' primer from a commercially supplied 3' RACE kit (Life Technologies, Gaithersburg, MD, Cat. No. 18373-019)

TABLE 5

	3' RACE Primers (5'-3')	Seq ID No.
PHN 11810	AAC ATG CGG TGG AGC TTT G	17
PHN 11811	CAU CAU CAU CAU CAT TCG CAT CGA GGT AAG	18

The first round of PCR amplification using the outside gene specific primer (GSP) PHN11810 and the oligo dT based 3' primer resulted in no visible DNA bands. The inside GSP PHN11811 and the oligo dT based 3' primer were then used for a second round of amplification on the same sample. A large number of bands appeared, some of which stained intensely with ethidium bromide and some which did not. The prominent bands were 0.4, 0.8 and 1.3 kb in size. This experiment was set up using 5' and 3' primers with custom ends which only allow ligation of DNA fragments amplified by both. This method permitted the reaction to be used in the ligation protocol without further purification or characterization of the DNA fragments. All three of the prominent bands described above were ligated into pAMP1 (Life Technologies, Cat. No., 18384-016), transformed into DH5α cells (Life Technologies, Cat. No. 18263-12), cloned and sequenced. The 0.4 kb band was found to encode an amino acid sequence having homology to the APOXD sequence data of Table 1.

5' RACE

Total RNA was reverse transcribed with commercially available components and a set of oligo dT-based primers ending in G, C or A which are collectively

termed Bam T17V (5' CGC GGA TCC GT_{17} V) 3') [Seq ID No. 19] These primers are disclosed in published PCT Application No. US96/08582. First strand cDNA was oligo dC-tailed and then column purified using commercially available components. (Life Technologies, Gaithersburg). The product of this reaction was then used in PCR with primer set Bam G13H, an equimolar mixture of oligo dG primers ending in A, C, or T (5' TAA GGA TCC TG_{13} H 3') [Seq. ID NO: 20], and a second gene specific primer, PHN 11813 [Seq ID No. 21]. Amplified products were characterized by Southern analysis using the protocol as described in Ausubel, et al. (eds.), 1989, *Current Protocols in Molecular Biology*, pages 2.0.1 - 2.12.5.

Hybridization of the 5' RACE product was done using the PCR amplified genomic DNA fragment (Table 4) as a radiolabeled probe. A 0.6 kb band was amplified by this reaction and was strongly labeled with the probe. No other bands appeared. This 0.6 kb band was ligated into the PCR II vector using the TA-cloning procedure, transformed into DH5I, cloned and sequenced. The DNA sequence analysis of the 0.6 kb PCR fragment showed it was homologous to the APOXD sequence data shown in Table 2.

TABLE 6

	5' RACE Primers	SEQ ID No
Bam T17V	5' CGC GGA TCC GT_{17}V 3'	19
Bam G13H	5' TAA GGA TCC TG_{13}H 3'	20
PHN 11813	5' CAU CAU CAU CAU TAC CTC GAT GCG AAT GTG 3'	21

IUPAC Symbols: V=G,C, or A; H=A, T, or C.

PCR For Full Length

The 5' and 3' RACE products were sequenced to their ends as determined by the initiating methionine and the poly-A tail respectively. DNA sequence at each end was analyzed by Oligo 4.0 for oligonucleotide primer design in preparation for PCR to obtain the complete gene.

Primer PHN 12566 designed to the 3' end of the sequence, was used to reverse transcribe total RNA. Primers PHN 12565 and PHN 12567 were used to amplify

first strand cDNA. The PCR amplified band was ligated into PCR II using the TA cloning kit (In Vitrogen; San Diego, CA) then transformed into DH5I, cloned, and sequenced.

TABLE 7

	Full Length cDNA Primers (5'→3')	SEQ. ID No.
PHN 12566	CGA TGA TAT CAG CAA AAT ACA CGC GTA	22
PHN 12565	GTC AGG ATC CCG CTT CAT CCC CAT CC	23
PHN 12567	CAT GAT ATC CTA CTC ACT TGG GCT CCG	24

A 1.4 kb band was amplified which stained very intensely with ethidium bromide. Other, smaller bands were present, but clearly, the 1.4 kb band was prominent. This band was sequenced and subjected to open reading frame analysis. All of the protein fragments originally sequenced (Table 2), were found in the deduced amino acid sequence of this PCR product.

Southern analysis was performed on genomic DNA using the 1.4 kb cDNA as a radiolabeled probe. Only one band hybridized, suggesting that the gene is a single copy and unique in the *A. phoenices* genome.

Table 1 (pages 4-7) shows the full length cDNA sequence [Seq ID No:1] and deduced amino acid sequence [Seq ID No:2] of the *A. phoenices* oxalate decarboxylase gene as amplified, using PCR primers PHN 12565 and PHN 12567. The underlined amino acid sequences were represented in the original protein sequence analysis data (Table 2). The protein sequence encoded by the full length cDNA includes a pre-protein, amino acid residues 27-458 [Seq ID No:4], and a mature protein, amino acid residues 50-458 [Seq ID No:5].

Example 2

Transformed plant tissue degrades oxalate

CaMV35S/O'/APOXD

The insert of pPHP9685 (1.4 kb APOXD cDNA in pCR II) was placed into a cloning vector intermediate (pLitmus 28, New England Biolabs) between a plant

expressible promoter and 3' region as shown in the construction diagrams of Figure 3. The upstream region consists of a cauliflower mosaic virus 35S promoter with a duplicated enhancer region (2X35S; bases -421 to -90 and -421 to +2, Gardner, et al., 1985, *Nucleic Acids Res.* 9:2871-2888) with a flanking 5' *Not*1 site and a 3' *Pst* site, and Ω' RNA leader sequence. The 3' region is from potato proteinase inhibitor II. These are described in Bidney, et al., 1992, *Plant Mol. Biol.* 18:301-313. The 2X CaMV 35S promoter is described in Odell, et al., *Nature* 313:810-812.

The plant-expressible APOXD gene cassette was then isolated from the cloning intermediate and ligated into the ALS::NPT II:: PIN II-containing pBIN19 construct, pPHP8110. Plasmid pPHP8110 was created from pBIN 19 (Bevan, 1984, *Nucleic Acids Res.* 12:8711-8721) by replacing the NOS::NPTII::NOS gene cassette in pBIN19 with an ALS::NPTII::PINII cassette. As shown in Figure 3, pPHP8110 is a derivative of pBIN19 containing the NPT II gene, the aminoglycoside-3'-O-phosphotransferase coding sequence, bases 1551 to 2345 from *E.coli* transposon TN5 (Genbank Accession Number V00004, Beck, et al., 1982, *Gene* 19:327-336). The second amino acid was modified from an isoleucine to a valine in order to create a *Nco* I restriction site which was used to make a translational fusion with the ALS promoter (see copending U.S. Patent Application Serial No. 08/409,297). pPHP8110 further contains the potato proteinase inhibitor II terminator (PIN II) bases 2-310, as described in An, et al., 1989, *Plant Cell* 1:115-122.

As shown in Figure 4, the resultant plasmid, pPHP9723, carries the APOXD gene construct, together with the NPTII gene for selection of transgenic plant cells, positioned between *Agrobacterium* T-DNA borders.

Germin/APOXD

A second APOXD cDNA containing plasmid was constructed using the methods described above for producing pPHP 9723. In the second construct, the APOXD fungal signal and presequence (49 amino acids) were replaced with a plant signal sequence obtained from the 5' end of an enzyme subunit of wheat oxalate oxidase. (Lane, et al., 1991, *J. Biol. Chem.* 266:10461.) This was accomplished by designing primers that were homologous to the Germin signal sequence, and having extensions to provide the addition of a *Sal* I restriction site at the 5' end and APOXD 5' sequence followed by a *Nru* I site at

the 3' end. The primers were used to amplify the Germin signal sequence and are shown below in Table 8.

Table 8

	Germin Signal Sequence Primers (5'-3')	Seq ID No.
PHN 13418	GAT GAC GCA CAA TCC CAC TAT CCT TCG CAA GAC CCT TC	25
PHN 13419	GGTT TCG CGATGA TCT GGGG TG AAA GG CTT AT CCT GGG TAG CC AAAA CAG CT GGAG	26

The amplified Germin signal sequence product [Seq ID NO:27] shown below in Table 9, and a vector containing the full length APOXD cDNA (pPHP9648) were each digested with *Sal* I and *Nru* I. A ligation reaction was set up with the digested fragments to form a Germin signal sequence - APOXD coding sequence fusion construct. Clones of the correct size were sequenced to verify correct results.

As shown in Table 9, the *Sal*I/*Nru*I cut Germin SS - containing sequence also contained modified APOXD codons matched to fill in the *Nru*I-cut APOXD sequence. The Germin signal sequence [Seq. ID No: 28] is shown in lower case.

Table 9

Amplified Germin Signal/APOXD Sequence*

```
  1  GCAGCTTATT TTTACAACAA TTACCAACAA CAACAAACAA AAACAACAT
                                 SalI                start
 51  TACAATTACT ATTTACAATT ACAGTCGACC CGGGATCC atg ggt tac

 98  tca aag acc ttg gtt gct ggt ttg ttc gct atg ttg ttg

137  ttg gct cca gct gtt ttg gct acc ICAG GAT AAG CCT TTC
                     NruI
176  ACC CCA GAT CAT CGC GA CCCCTATG ATCACAAGGT GGATGCGATC

221  GGGGAAGGCC ATGAGCCCTT GCCCTGGCGC ATGGGAGATG GAGCCACCAT

271  CATGGGACCC CGCAACAAGG ACCGTGAGCG CCAGAACCCC GACATGCTCC
```

```
311  GTCCTCCGAG CACCGACCAT GGCAACATGC CGAACATGCG GTGGAGCTTT

361  GCTGACTCCC ACATTCGCAT CGAGGAGGGC GGCTGGACAC GCCAGACTAC

411  CGTACGCGAG CTGCCAACGA GCAAGGAGCT TGCGGGTGTA AACATGCGCC

461  TCGATGAGGG TGTCATCCGC GAGTTGCACT GGCATCGA
```

*The *Sal*I (GTCGAC) and *Nru*I (TCGCGA) restriction sites are underlined, the Germin signal sequence is in lower case, with the Germin start site in bold. APOXD sequences modified in the PCR primer design are shown in bold.

This fusion gene was placed in the binary T-DNA plasmid to produce plasmid pPHP9762 carrying the fusion gene and the plant expressible NPTII gene positioned between *Agrobacterium* T-DNA borders, as described above.

Agrobacterium tumefaciens strain EHA105 (as described in Hood, et al., 1993, *Transgen. Res.* 2:208-218) was transformed with kanamycin resistant binary T-DNA vectors carrying the different versions of APOXD. Transformation was accomplished by the freeze-thaw method of Holsters, et al., 1978, *Mol. Gen. Genetics* 1:181-7. The transformed isolates were selected on solidified 60A (YEP; 10 g/l yeast extract, 10 g/l bactopeptone, 5 g/l NaCl, pH 7.0) medium with 50 mg/l kanamycin. Transformed bacteria were cultured in liquid culture of YEP medium containing 50 mg/l kanamycin, to log phase growth ($O.D._{600}$ 0.5-1.0) for use in plant transformations. Binary plasmids were re-isolated from transformed *Agrobacterium* to verify that integrity was maintained throughout the transformation procedures.

Sunflower leaf discs were obtained by harvesting leaves which were not fully expanded, sterilizing the surface in 20% bleach with TWEEN 20, and punching discs out of the leaf with a paper punch. *Agrobacterium* suspensions were centrifuged and resuspended in inoculation medium (12.5 μM MES buffer, pH 5.7, 1 g/l NH_4Cl, 0.3 g/l $MgSO_4$) to a calculated OD_{600} of 0.75 as described in Malone-Schoneberg, et al., 1994, *Plant Science* 103:199-207. Leaf discs were inoculated in the resuspended *Agrobacterium* for 10 minutes then blotted on sterile filter paper.

The tissue and bacteria were co-cultivated on 527 for 3 days, then transferred to 527E medium for the selection of transgenic plant cells. After 2 weeks of

culture, the transgenic callus nodes were removed from the leaf disc and subcultured on fresh 527E medium. A number of subcultures were repeated prior to the assay of the callus tissue for enzyme activity.

To assay for enzyme activity, callus was harvested, snap frozen in liquid nitrogen, lyophilized to dryness and powdered. A quantity of 0.75 mg of powder from each prepared callus line was added to 1.0 ml reaction mixture (900 µl 200 mM $NaPO_4$, pH 5.0, 100 µl 10 mM Na-oxalate pH 5.0). The reaction proceeded for 3 hours at room temperature and was stopped by the addition of 150 µl of 1M TRIS-HCl, pH 7.0. Each sample was spun at 14,000 rpm for one minute and 1 ml was removed to a cuvette. One hundred (100) µl of 9-NAD (6.6 mg/ml stock) and 50 µl formate dehydrogenase (4.0 mg/ml stock) were added and the increase in absorbance was measured at 340 nm. A slope was generated for each sample as well as for a formate standard curve. Assay results were reported as µM oxalate metabolized /mg powder.

The results of the leaf disk assay are shown below in Table 10, and demonstrate that the APOXD gene sequence produces enzyme that is active in transgenic callus. No activity was seen in control callus, or callus transformed with the native APOXD gene (pPHP 9723).

Table 10

Oxalate Decarboxylase Activity in Transgenic Sunflower Tissue

Callus Line	Binary Vector	Activity µM oxalate/min/mg
SMF3	None	0
9723 -1	pPHP 9723	0
-2	pPHP 9723	0
-3	pPHP 9723	0
9762-1	pPHP 9762	1.35
-2	pPHP 9762	1.40
-3	pPHP 9762	0.87
-4	pPHP 9762	0.81
-5	pPHP 9762	0.81
-6	pPHP 9762	0.90

Example 3

Transgenic Sunflower Plants Expressing APOXD

Sunflower plants were transformed using a basic transformation protocol involving a combination of wounding by particle bombardment, followed by use of *Agrobacterium* for DNA delivery, as described by Bidney, et al. *Plant Mol. Biol.* 18:301-313. The plasmid pPHP9762, as described above for Example 2 and shown in Figure 5, was used in these experiments. pPHP9762 contains the APOXD gene with the fungal signal and presequence replaced with the Germin signal sequence and a plant expressible NPTII gene which provides kanamycin resistance to transgenic plant tissues.

Procedures for preparation of *Agrobacterium* and preparation of particles for wounding are described in Bidney, et al., 1992, *Plant Mol. Biol.* 18:301-313. The Pioneer sunflower line SMF3, used in these experiments, is described in Burrus, et al., 1991, *Plant Cell Rep.* 10:161-166. The *Agrobacterium* strain used in these experiments, EHA 105. Procedures for use of the helium gun, intact meristem preparation, tissue culture and co-cultivation conditions, as well as recovery of transgenic plants, are described in Bidney, et al., 1992, *Plant Mol. Biol.* 18:301-313.

Sunflower explants were prepared by imbibing seed overnight, removing the cotyledons and radical tip, then culturing overnight on medium containing plant growth regulators. Primary leaves were then removed and explants arranged in the center of a petri plate for bombardment. The PDS 1000 helium-driven particle bombardment device (Bio-Rad) was used with 600 psi rupture discs and a vacuum of 26 inches, Hg to bombard meristem explants twice on the highest shelf position. Following bombardment, log phase *Agrobacterium* cultures transformed with the APOXD-plasmid pPHP 9762, as described for Example 2, were centrifuged and resuspended at a calculated OD600 (vis) of 4.0 in inoculation buffer. *Agrobacterium* was then dropped onto the meristem explants using a fine tipped pipettor. Inoculated explants were co-cultured for three days then transferred to medium containing 50 mg/l kanamycin and 250 mg/l cefotaxime for selection. Explants were cultured on this medium for two weeks then transferred to the same medium, but lacking kanamycin. Green, kanamycin-resistant shoots were recovered to the greenhouse and assayed by an NPTII ELISA assay to verify transformation. Oxalate decarboxylase enzyme assays are performed on these plants and/or progeny to confirm the expression of APOXD.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SCELONGE, CHRISTOPHER
BIDNEY, DENNIS

(ii) TITLE OF THE INVENTION:
GENE ENCODING OXALATE DECARBOXYLASE FROM ASPERGILLUS PHOENICES

(iii) NUMBER OF SEQUENCES: 29

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(E) COUNTRY: USA
(F) ZIP: 55402

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: March 21, 1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

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(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1437 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 24...1397
(D) OTHER INFORMATION:

(A) NAME/KEY: sig_peptide
(B) LOCATION: 24...101
(D) OTHER INFORMATION:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 171...1397
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GGCTTGTCAG GATCCTTCCA AAG ATG CAG CTA ACC CTG CCA CCA CGT CAG CTG      53
                          Met Gln Leu Thr Leu Pro Pro Arg Gln Leu
                           1               5                  10

TTG CTG AGT TTC GCG ACC GTG GCC GCC CTC CTT GAT CCA AGC CAT GGA      101
Leu Leu Ser Phe Ala Thr Val Ala Ala Leu Leu Asp Pro Ser His Gly
               15                  20                  25

GGC CCG GTC CCT AAC GAA GCG TAC CAG CAA CTA CTG CAG ATT CCC GCC      149
Gly Pro Val Pro Asn Glu Ala Tyr Gln Gln Leu Leu Gln Ile Pro Ala
           30                  35                  40

TCA TCC CCA TCC ATT TTC TTC CAA GAC AAG CCA TTC ACC CCC GAT CAT      197
Ser Ser Pro Ser Ile Phe Phe Gln Asp Lys Pro Phe Thr Pro Asp His
       45                  50                  55

CGC GAC CCC TAT GAT CAC AAG GTG GAT GCG ATC GGG GAA GGC CAT GAG      245
Arg Asp Pro Tyr Asp His Lys Val Asp Ala Ile Gly Glu Gly His Glu
   60                  65                  70

CCC TTG CCC TGG CGC ATG GGA GAT GGA GCC ACC ATC ATG GGA CCC CGC      293
Pro Leu Pro Trp Arg Met Gly Asp Gly Ala Thr Ile Met Gly Pro Arg
75                 80                  85                  90
```

```
AAC AAG GAC CGT GAG CGC CAG AAC CCC GAC ATG CTC CGT CCT CCG AGC      341
Asn Lys Asp Arg Glu Arg Gln Asn Pro Asp Met Leu Arg Pro Pro Ser
                95                  100                 105

ACC GAC CAT GGC AAC ATG CCG AAC ATG CGG TGG AGC TTT GCT GAC TCC      389
Thr Asp His Gly Asn Met Pro Asn Met Arg Trp Ser Phe Ala Asp Ser
            110                 115                 120

CAC ATT CGC ATC GAG GAG GGC GGC TGG ACA CGC CAG ACT ACC GTA CGC      437
His Ile Arg Ile Glu Glu Gly Gly Trp Thr Arg Gln Thr Thr Val Arg
        125                 130                 135

GAG CTG CCA ACG AGC AAG GAG CTT GCG GGT GTA AAC ATG CGC CTC GAT      485
Glu Leu Pro Thr Ser Lys Glu Leu Ala Gly Val Asn Met Arg Leu Asp
    140                 145                 150

GAG GGT GTC ATC CGC GAG TTG CAC TGG CAT CGA GAA GCA GAG TGG GCG      533
Glu Gly Val Ile Arg Glu Leu His Trp His Arg Glu Ala Glu Trp Ala
155                 160                 165                 170

TAT GTG CTG GCC GGA CGT GTA CGA GTG ACT GGC CTT GAC CTG GAG GGA      581
Tyr Val Leu Ala Gly Arg Val Arg Val Thr Gly Leu Asp Leu Glu Gly
                175                 180                 185

GGC AGC TTC ATC GAC GAC CTA GAA GAG GGT GAC CTC TGG TAC TTC CCA      629
Gly Ser Phe Ile Asp Asp Leu Glu Glu Gly Asp Leu Trp Tyr Phe Pro
            190                 195                 200

TCG GGC CAT CCC CAT TCG CTT CAG GGT CTC AGT CCT AAT GGC ACC GAG      677
Ser Gly His Pro His Ser Leu Gln Gly Leu Ser Pro Asn Gly Thr Glu
        205                 210                 215

TTC TTA CTG ATC TTC GAC GAT GGA AAC TTT TCC GAG GAG TCA ACG TTC      725
Phe Leu Leu Ile Phe Asp Asp Gly Asn Phe Ser Glu Glu Ser Thr Phe
    220                 225                 230

TTG TTG ACC GAC TGG ATC GCA CAT ACA CCC AAG TCT GTC CTC GCC GGA      773
Leu Leu Thr Asp Trp Ile Ala His Thr Pro Lys Ser Val Leu Ala Gly
235                 240                 245                 250

AAC TTC CGC ATG CGC CCA CAA ACA TTT AAG AAC ATC CCA CCA TCT GAA      821
Asn Phe Arg Met Arg Pro Gln Thr Phe Lys Asn Ile Pro Pro Ser Glu
                255                 260                 265

AAG TAC ATC TTC CAG GGC TCT GTC CCA GAC TCT ATT CCC AAA GAG CTC      869
Lys Tyr Ile Phe Gln Gly Ser Val Pro Asp Ser Ile Pro Lys Glu Leu
            270                 275                 280

CCC CGC AAC TTC AAA GCA TCC AAG CAG CGC TTC ACG CAT AAG ATG CTC      917
Pro Arg Asn Phe Lys Ala Ser Lys Gln Arg Phe Thr His Lys Met Leu
        285                 290                 295

GCT CAA AAA CCC GAA CAT ACC TCT GGC GGA GAG GTG CGC ATC ACA GAC      965
Ala Gln Lys Pro Glu His Thr Ser Gly Gly Glu Val Arg Ile Thr Asp
```

```
    300                 305                 310

TCG TCC AAC TTT CCC ATC TCC AAG ACG GTC GCG GCC GCC CAC CTG ACC     1013
Ser Ser Asn Phe Pro Ile Ser Lys Thr Val Ala Ala Ala His Leu Thr
315                 320                 325                 330

ATT AAC CCG GGT GCT ATC CGG GAG ATG CAC TGG CAT CCC AAT GCG GAT     1061
Ile Asn Pro Gly Ala Ile Arg Glu Met His Trp His Pro Asn Ala Asp
                335                 340                 345

GAA TGG TCC TAC TTT AAG CGC GGT CGG GCG CGA GTG ACT ATC TTC GCT     1109
Glu Trp Ser Tyr Phe Lys Arg Gly Arg Ala Arg Val Thr Ile Phe Ala
            350                 355                 360

GCT GAA GGT AAT GCT CGT ACG TTC GAC TAC GTA GCG GGA GAT GTG GGC     1157
Ala Glu Gly Asn Ala Arg Thr Phe Asp Tyr Val Ala Gly Asp Val Gly
        365                 370                 375

ATT GTT CCT CGC AAC ATG GGT CAT TTC ATT GAG AAC CTT AGT GAT GAC     1205
Ile Val Pro Arg Asn Met Gly His Phe Ile Glu Asn Leu Ser Asp Asp
    380                 385                 390

GAG AGG TCG AGG TGT TGG AAA TCT TCC GGG CGG ACC GAT TCC GGG ACT     1253
Glu Arg Ser Arg Cys Trp Lys Ser Ser Gly Arg Thr Asp Ser Gly Thr
395                 400                 405                 410

TTT CTT TGT TCC AGT GGA TGG GAG AGA CGC CGC AGC GGA TGG TGG CAG     1301
Phe Leu Cys Ser Ser Gly Trp Glu Arg Arg Arg Ser Gly Trp Trp Gln
                415                 420                 425

AGC ATG TGT TTA AGG ATG ATC CAG ATG CGG CCA GGG AGT TCC TTA AGA     1349
Ser Met Cys Leu Arg Met Ile Gln Met Arg Pro Gly Ser Ser Leu Arg
            430                 435                 440

GTG TGG AGA GTG GGG AGA AGG ATC CAA TTC GGA GCC CAA GTG AGT AGA T   1398
Val Trp Arg Val Gly Arg Arg Ile Gln Phe Gly Ala Gln Val Ser Arg
        445                 450                 455

GAGGTTCTAC GCGTGTATTT TGCTGATATC ATCGAAGCC                          1437
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 458 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Gln Leu Thr Leu Pro Pro Arg Gln Leu Leu Leu Ser Phe Ala Thr
 1               5                  10                  15
Val Ala Ala Leu Leu Asp Pro Ser His Gly Gly Pro Val Pro Asn Glu
            20                  25                  30
Ala Tyr Gln Gln Leu Leu Gln Ile Pro Ala Ser Ser Pro Ser Ile Phe
        35                  40                  45
Phe Gln Asp Lys Pro Phe Thr Pro Asp His Arg Asp Pro Tyr Asp His
    50                  55                  60
Lys Val Asp Ala Ile Gly Glu Gly His Glu Pro Leu Pro Trp Arg Met
65                  70                  75                  80
Gly Asp Gly Ala Thr Ile Met Gly Pro Arg Asn Lys Asp Arg Glu Arg
                85                  90                  95
Gln Asn Pro Asp Met Leu Arg Pro Pro Ser Thr Asp His Gly Asn Met
            100                 105                 110
Pro Asn Met Arg Trp Ser Phe Ala Asp Ser His Ile Arg Ile Glu Glu
        115                 120                 125
Gly Gly Trp Thr Arg Gln Thr Thr Val Arg Glu Leu Pro Thr Ser Lys
    130                 135                 140
Glu Leu Ala Gly Val Asn Met Arg Leu Asp Glu Gly Val Ile Arg Glu
145                 150                 155                 160
Leu His Trp His Arg Glu Ala Glu Trp Ala Tyr Val Leu Ala Gly Arg
                165                 170                 175
Val Arg Val Thr Gly Leu Asp Leu Glu Gly Gly Ser Phe Ile Asp Asp
            180                 185                 190
Leu Glu Glu Gly Asp Leu Trp Tyr Phe Pro Ser Gly His Pro His Ser
        195                 200                 205
Leu Gln Gly Leu Ser Pro Asn Gly Thr Glu Phe Leu Leu Ile Phe Asp
    210                 215                 220
Asp Gly Asn Phe Ser Glu Glu Ser Thr Phe Leu Leu Thr Asp Trp Ile
225                 230                 235                 240
Ala His Thr Pro Lys Ser Val Leu Ala Gly Asn Phe Arg Met Arg Pro
                245                 250                 255
Gln Thr Phe Lys Asn Ile Pro Pro Ser Glu Lys Tyr Ile Phe Gln Gly
            260                 265                 270
Ser Val Pro Asp Ser Ile Pro Lys Glu Leu Pro Arg Asn Phe Lys Ala
        275                 280                 285
Ser Lys Gln Arg Phe Thr His Lys Met Leu Ala Gln Lys Pro Glu His
    290                 295                 300
Thr Ser Gly Gly Glu Val Arg Ile Thr Asp Ser Ser Asn Phe Pro Ile
305                 310                 315                 320
Ser Lys Thr Val Ala Ala Ala His Leu Thr Ile Asn Pro Gly Ala Ile
                325                 330                 335
Arg Glu Met His Trp His Pro Asn Ala Asp Glu Trp Ser Tyr Phe Lys
            340                 345                 350
Arg Gly Arg Ala Arg Val Thr Ile Phe Ala Ala Glu Gly Asn Ala Arg
        355                 360                 365
Thr Phe Asp Tyr Val Ala Gly Asp Val Gly Ile Val Pro Arg Asn Met
    370                 375                 380
Gly His Phe Ile Glu Asn Leu Ser Asp Asp Glu Arg Ser Arg Cys Trp
385                 390                 395                 400
Lys Ser Ser Gly Arg Thr Asp Ser Gly Thr Phe Leu Cys Ser Ser Gly
                405                 410                 415
```

```
Trp Glu Arg Arg Arg Ser Gly Trp Trp Gln Ser Met Cys Leu Arg Met
            420                 425                 430
Ile Gln Met Arg Pro Gly Ser Ser Leu Arg Val Trp Arg Val Gly Arg
        435                 440                 445
Arg Ile Gln Phe Gly Ala Gln Val Ser Arg
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Gln Leu Thr Leu Pro Pro Arg Gln Leu Leu Leu Ser Phe Ala Thr
 1               5                  10                  15
Val Ala Ala Leu Leu Asp Pro Ser His Gly
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 432 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Gly Pro Val Pro Asn Glu Ala Tyr Gln Gln Leu Leu Gln Ile Pro Ala
 1               5                  10                  15
Ser Ser Pro Ser Ile Phe Phe Gln Asp Lys Pro Phe Thr Pro Asp His
            20                  25                  30
Arg Asp Pro Tyr Asp His Lys Val Asp Ala Ile Gly Glu Gly His Glu
        35                  40                  45
Pro Leu Pro Trp Arg Met Gly Asp Gly Ala Thr Ile Met Gly Pro Arg
    50                  55                  60
Asn Lys Asp Arg Glu Arg Gln Asn Pro Asp Met Leu Arg Pro Pro Ser
65                  70                  75                  80
Thr Asp His Gly Asn Met Pro Asn Met Arg Trp Ser Phe Ala Asp Ser
                85                  90                  95
His Ile Arg Ile Glu Glu Gly Gly Trp Thr Arg Gln Thr Thr Val Arg
            100                 105                 110
Glu Leu Pro Thr Ser Lys Glu Leu Ala Gly Val Asn Met Arg Leu Asp
        115                 120                 125
```

```
Glu Gly Val Ile Arg Glu Leu His Trp His Arg Glu Ala Glu Trp Ala
    130                 135                 140
Tyr Val Leu Ala Gly Arg Val Arg Val Thr Gly Leu Asp Leu Glu Gly
145                 150                 155                 160
Gly Ser Phe Ile Asp Asp Leu Glu Glu Gly Asp Leu Trp Tyr Phe Pro
                165                 170                 175
Ser Gly His Pro His Ser Leu Gln Gly Leu Ser Pro Asn Gly Thr Glu
            180                 185                 190
Phe Leu Leu Ile Phe Asp Asp Gly Asn Phe Ser Glu Glu Ser Thr Phe
        195                 200                 205
Leu Leu Thr Asp Trp Ile Ala His Thr Pro Lys Ser Val Leu Ala Gly
    210                 215                 220
Asn Phe Arg Met Arg Pro Gln Thr Phe Lys Asn Ile Pro Pro Ser Glu
225                 230                 235                 240
Lys Tyr Ile Phe Gln Gly Ser Val Pro Asp Ser Ile Pro Lys Glu Leu
                245                 250                 255
Pro Arg Asn Phe Lys Ala Ser Lys Gln Arg Phe Thr His Lys Met Leu
            260                 265                 270
Ala Gln Lys Pro Glu His Thr Ser Gly Gly Glu Val Arg Ile Thr Asp
        275                 280                 285
Ser Ser Asn Phe Pro Ile Ser Lys Thr Val Ala Ala Ala His Leu Thr
    290                 295                 300
Ile Asn Pro Gly Ala Ile Arg Glu Met His Trp His Pro Asn Ala Asp
305                 310                 315                 320
Glu Trp Ser Tyr Phe Lys Arg Gly Arg Ala Arg Val Thr Ile Phe Ala
                325                 330                 335
Ala Glu Gly Asn Ala Arg Thr Phe Asp Tyr Val Ala Gly Asp Val Gly
            340                 345                 350
Ile Val Pro Arg Asn Met Gly His Phe Ile Glu Asn Leu Ser Asp Asp
        355                 360                 365
Glu Arg Ser Arg Cys Trp Lys Ser Ser Gly Arg Thr Asp Ser Gly Thr
    370                 375                 380
Phe Leu Cys Ser Ser Gly Trp Glu Arg Arg Arg Ser Gly Trp Trp Gln
385                 390                 395                 400
Ser Met Cys Leu Arg Met Ile Gln Met Arg Pro Gly Ser Ser Leu Arg
                405                 410                 415
Val Trp Arg Val Gly Arg Arg Ile Gln Phe Gly Ala Gln Val Ser Arg
            420                 425                 430
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 409 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Gln Asp Lys Pro Phe Thr Pro Asp His Arg Asp Pro Tyr Asp His Lys
 1               5                  10                  15
```

Val Asp Ala Ile Gly Glu Gly His Glu Pro Leu Pro Trp Arg Met Gly
20 25 30
Asp Gly Ala Thr Ile Met Gly Pro Arg Asn Lys Asp Arg Glu Arg Gln
35 40 45
Asn Pro Asp Met Leu Arg Pro Pro Ser Thr Asp His Gly Asn Met Pro
50 55 60
Asn Met Arg Trp Ser Phe Ala Asp Ser His Ile Arg Ile Glu Glu Gly
65 70 75 80
Gly Trp Thr Arg Gln Thr Thr Val Arg Glu Leu Pro Thr Ser Lys Glu
85 90 95
Leu Ala Gly Val Asn Met Arg Leu Asp Glu Gly Val Ile Arg Glu Leu
100 105 110
His Trp His Arg Glu Ala Glu Trp Ala Tyr Val Leu Ala Gly Arg Val
115 120 125
Arg Val Thr Gly Leu Asp Leu Glu Gly Gly Ser Phe Ile Asp Asp Leu
130 135 140
Glu Glu Gly Asp Leu Trp Tyr Phe Pro Ser Gly His Pro His Ser Leu
145 150 155 160
Gln Gly Leu Ser Pro Asn Gly Thr Glu Phe Leu Leu Ile Phe Asp Asp
165 170 175
Gly Asn Phe Ser Glu Glu Ser Thr Phe Leu Leu Thr Asp Trp Ile Ala
180 185 190
His Thr Pro Lys Ser Val Leu Ala Gly Asn Phe Arg Met Arg Pro Gln
195 200 205
Thr Phe Lys Asn Ile Pro Pro Ser Glu Lys Tyr Ile Phe Gln Gly Ser
210 215 220
Val Pro Asp Ser Ile Pro Lys Glu Leu Pro Arg Asn Phe Lys Ala Ser
225 230 235 240
Lys Gln Arg Phe Thr His Lys Met Leu Ala Gln Lys Pro Glu His Thr
245 250 255
Ser Gly Gly Glu Val Arg Ile Thr Asp Ser Ser Asn Phe Pro Ile Ser
260 265 270
Lys Thr Val Ala Ala Ala His Leu Thr Ile Asn Pro Gly Ala Ile Arg
275 280 285
Glu Met His Trp His Pro Asn Ala Asp Glu Trp Ser Tyr Phe Lys Arg
290 295 300
Gly Arg Ala Arg Val Thr Ile Phe Ala Ala Glu Gly Asn Ala Arg Thr
305 310 315 320
Phe Asp Tyr Val Ala Gly Asp Val Gly Ile Val Pro Arg Asn Met Gly
325 330 335
His Phe Ile Glu Asn Leu Ser Asp Asp Glu Arg Ser Arg Cys Trp Lys
340 345 350
Ser Ser Gly Arg Thr Asp Ser Gly Thr Phe Leu Cys Ser Ser Gly Trp
355 360 365
Glu Arg Arg Arg Ser Gly Trp Trp Gln Ser Met Cys Leu Arg Met Ile
370 375 380
Gln Met Arg Pro Gly Ser Ser Leu Arg Val Trp Arg Val Gly Arg Arg
385 390 395 400
Ile Gln Phe Gly Ala Gln Val Ser Arg
405

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Gln Asp Lys Pro Phe Thr Pro Asp His Arg Asp Pro Tyr Asp His Lys
 1               5                  10                  15
Val Asp Ala Ile Gly Glu Xaa His Glu Pro Leu
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Val Ile Arg Glu Leu His Trp His Arg Glu Ala Gly
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Arg Leu Asp Glu Gly Val Ile Arg Glu Leu His Cys His Arg Glu Ala
 1               5                  10                  15
Glu
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Tyr Phe Lys Arg Gly Arg Ala Arg Tyr Thr Ile Phe Ala Ala Glu
1 5 10 15
Gly Asn Ala Arg
20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Ala His Thr Pro Pro Ser Val Leu Ala Gly Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CAUCAUCAUC AUCCATGGGA YCAYCGNGAY CCYTA 35

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CUACUACUAC UAAGGCCTGT GNRRYTCNCG DATVA 35

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CACCATGGTA CGATCACAAG GT 22

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCAACGTGAC CGTTCCGGAC T 21

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 440 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 4...198
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
ACG ATC ACA AGG TGG ATG CGA TCG GGG AAG GCC ATG AGC CCT TGC CCT      48
    Ile Thr Arg Trp Met Arg Ser Gly Lys Ala Met Ser Pro Cys Pro
     1               5                  10                  15

GGC GCA TGG GAG ATG GAG CCA CCA TCA TGG GAC CCC GCA ACA AGG ACC      96
Gly Ala Trp Glu Met Glu Pro Pro Ser Trp Asp Pro Ala Thr Arg Thr
                20                  25                  30

GTG AGC GCC AGA ACC CCG ACA TGC TCC GTC CTC CGA GCA CCG ACC ATG     144
Val Ser Ala Arg Thr Pro Thr Cys Ser Val Leu Arg Ala Pro Thr Met
            35                  40                  45

GCA ACA TGC CGA ACA TGC GGT GGA GCT TTG CTG ACT CCC ACA TTC GCA     192
Ala Thr Cys Arg Thr Cys Gly Gly Ala Leu Leu Thr Pro Thr Phe Ala
        50                  55                  60

TCG AGG TAAGCCCTTC GAGGGTTTTG TGTACGACAA GCAAAATAGG CTAATGCACT GC   250
Ser Arg
    65

AGGAGGGCGG CTGGACACGC CAGACTACCG TACGCGAGCT GCCAACGAGC AAGGAGCTTG   310
CGGGTGTAAA CATGCGCCTC GATGAGGGTG TCATCCGCGA GTTGCACTGG CAAGGGCTGA   370
AGGCGAATTC CAGCACACTG GCGGCCGTTA CTAGTGGATC CGAGCTCGGT ACCAAGCTTG   430
ATGCATAGCT                                                          440
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Ile Thr Arg Trp Met Arg Ser Gly Lys Ala Met Ser Pro Cys Pro Gly
 1               5                  10                  15
Ala Trp Glu Met Glu Pro Pro Ser Trp Asp Pro Ala Thr Arg Thr Val
            20                  25                  30
Ser Ala Arg Thr Pro Thr Cys Ser Val Leu Arg Ala Pro Thr Met Ala
        35                  40                  45
Thr Cys Arg Thr Cys Gly Gly Ala Leu Leu Thr Pro Thr Phe Ala Ser
    50                  55                  60
Arg
65
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
AACATGCGGT GGAGCTTTG                                              19
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
CAUCAUCAUC AUCATTCGCA TCGAGGTAAG                                  30
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGCGGATCCG TTTTTTTTTT TTTTTTTV 28

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TAAGGATCCT GGGGGGGGGG GGGH 24

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAUCAUCAUC AUTACCTCGA TGCGAATGTG 30

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGATGATATC AGCAAAATAC ACGCGTAG 28

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTCAGGATCC CGCTTCATCC CCATCC 26

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CATGATATCC TACTCACTTG GGCTCCG 27

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GATGACGCAC AATCCCACTA TCCTTCGCAA GACCCTTC 38

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGTTTCGCGA TGATCTGGGG TGAAAGGCTT ATCCTGGGTA GCCAAAACAG CTGGAG 56

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 507 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCAGCTTATT TTTACAACAA TTACCAACAA CAACAAACAA AAACAACATT ACAATTACTA 60
TTTACAATTA CAGTCGACCC GGGATCCATG GGTTACTCAA AGACCTTGGT TGCTGGTTTG 120
TTCGCTATGT TGTTGTTGGC TCCAGCTGTT TTGGCTACCC AGGATAAGCC TTTCACCCCA 180
GATCATCGCG ACCCCTATGA TCACAAGGTG GATGCGATCG GGGAAGGCCA TGAGCCCTTG 240
CCCTGGCGCA TGGGAGATGG AGCCACCATC ATGGGACCCC GCAACAAGGA CCGTGAGCGC 300
CAGAACCCCG ACATGCTCCG TCCTCCGAGC ACCGACCATG GCAACATGCC GAACATGCGG 360
TGGAGCTTTG CTGACTCCCA CATTCGCATC GAGGAGGGCG GCTGGACACG CCAGACTACC 420
GTACGCGAGC TGCCAACGAG CAAGGAGCTT GCGGGTGTAA ACATGCGCCT CGATGAGGGT 480
GTCATCCGCG AGTTGCACTG GCATCGA 507

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ATGGGTTACT CAAAGACCTT GGTTGCTGGT TTGTTCGCTA TGTTGTTGTT GGCTCCAGCT 60
GTTTTGGCTA CC 72

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Trp Thr Arg Gln Thr Thr Val Arg Glu Leu Pro Thr Ser Lys Glu
1 5 10 15
Leu Ala Gly Val Asn Met Arg Leu Asp Glu Gly Val Ile Arg Glu Leu
20 25 30
His Trp

We Claim:

1. An isolated nucleic acid sequence encoding *Aspergillus phoenices* oxalate decarboxylase.

2. The nucleic acid sequence of claim 1, having the sequence of the *Aspergillus phoenices* insert of the plasmid ATCC No. _____.

3. An isolated nucleic acid sequence encoding an oxalate decarboxylase enzyme from *Aspergillus phoenices* and comprising at least the coding sequence of SEQ. ID NO:1 or variations thereof permitted by the degeneracy of the genetic code.

4. The nucleic acid sequence of claim 3, further comprising a plant signal sequence.

5. A vector for delivery of a nucleic acid sequence to a host cell, the vector comprising the nucleic acid sequence of claim 3.

6. A host cell containing the vector of claim 5.

7. A host cell transformed with the nucleic acid sequence of claim 3.

8. The host cell of claim 7, wherein the cell is a plant cell.

9. The host cell of claim 8, wherein the nucleic acid sequence further comprises a plant signal sequence.

10. The host cell of claim 9, wherein said plant signal sequence comprises the Germin signal sequence contained in SEQ ID NO:28.

11. The host cell of claim 8, wherein the plant is selected from the group consisting of sunflower, bean, canola, alfalfa, soybean, flax, safflower, peanut and clover.

12. A plant cell transformed with a nucleic acid sequence comprising at least the coding sequence of SEQ ID NO:1 or variations thereof permitted by the degeneracy of the genetic code.

13. A plant having stably incorporated within its genome a nucleic acid sequence comprising at least the coding sequence of SEQ ID NO:1 or variations thereof permitted by the degeneracy of the genetic code.

14. The plant of claim 13, wherein said nucleic acid sequence further comprises a plant signal sequence.

15. The plant of claim 14, wherein said plant signal sequence comprises the Germin signal sequence contained in SEQ ID NO:28.

16. A method for degrading oxalic acid comprising expressing in a plant an *Aspergillus phoenices* oxalic acid decarboxylase from a nucleic acid sequence comprising at least the coding sequence of SEQ ID NO:1 or variations thereof permitted by the degeneracy of the genetic code.

17. The method of claim 16, wherein said nucleic acid sequence is integrated into the plant's genome.

18. The method of claim 16, wherein said nucleic acid sequence further comprises a plant signal sequence.

19. The method of claim 18, wherein said plant signal sequence comprises the Germin signal sequence contained in SEQ ID NO:28.

20. The method of claim 16, wherein said plant is selected from the group consisting of sunflower, bean, canola, alfalfa, soybean, flax, safflower, peanut and clove.

21. The method of claim 20, wherein said plant is sunflower.

22. A method of identifying transformed plant cells using the toxin oxalic acid as a phytotoxic marker, comprising the steps of:

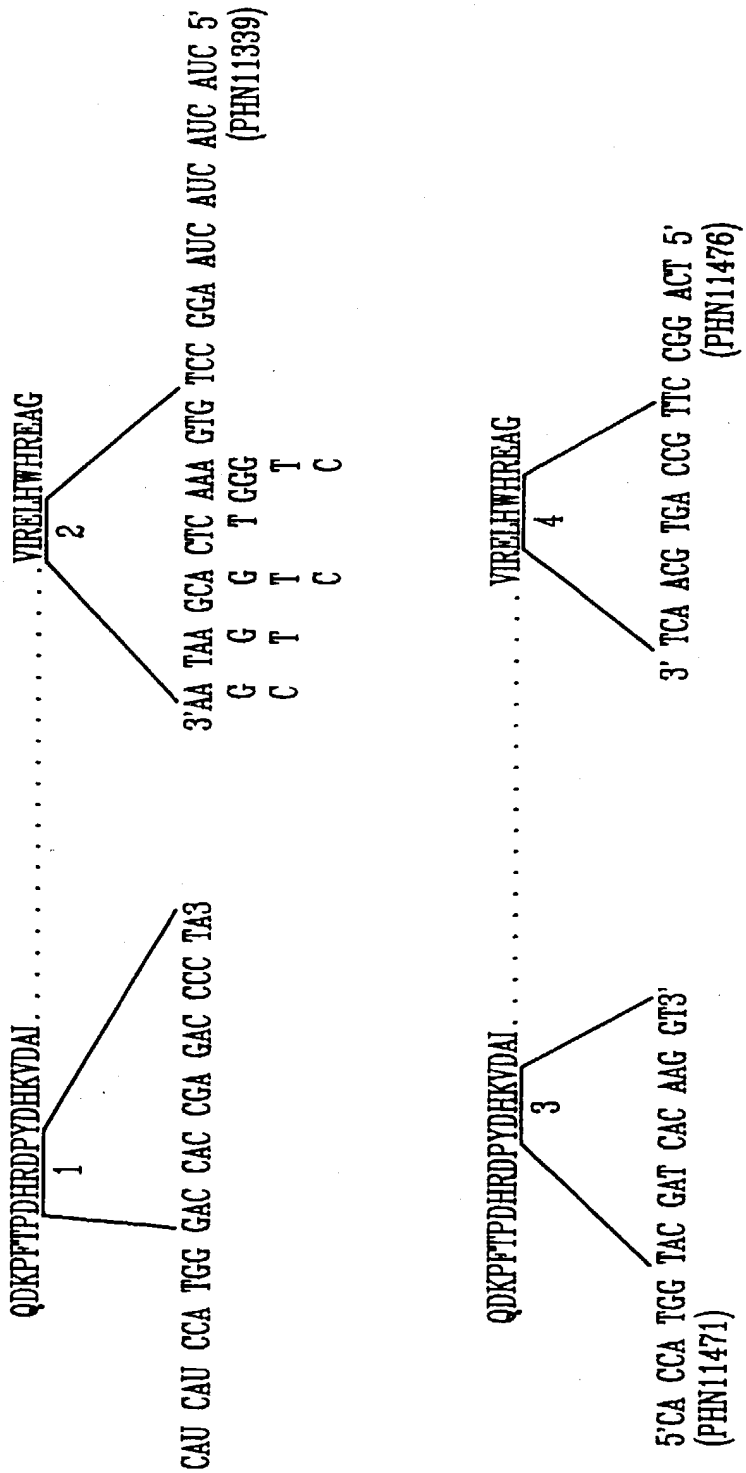
culturing cells or tissues from a selected target plant in a culture medium;

introducing into cells of the culture at least one copy of an expression cassette comprising a coding sequence of SEQ ID NO:1 operatively linked to an upstream transcription initiation sequence and a downstream polyadenylation sequence causing expression of the enzyme in the cells;

introducing oxalic acid into the culture medium; and

identifying transformed cells as the surviving cells in the oxalic acid-treated culture.

1/5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1
5'CAU CAU CAU CCA TGG GAC CAC CGA GAC CCC TA3
(PHN11337)
VIRELHWHREAG
2
3'AA TAA GCA CTC AAA GTG TCC GGA AUC AUC AUC AUC 5'
G G G T GGG
C T T C C
(PHN11339)
QDKPFTPDHRDPYDHKVDAI
3
5'CA CCA TGG TAC GAT CAC AAG GT3'
(PHN11471)
VIRELHWHREAG
4
3' TCA ACG TGA CCG TTC CGG ACT 5'
(PHN11476)

FIG. 1

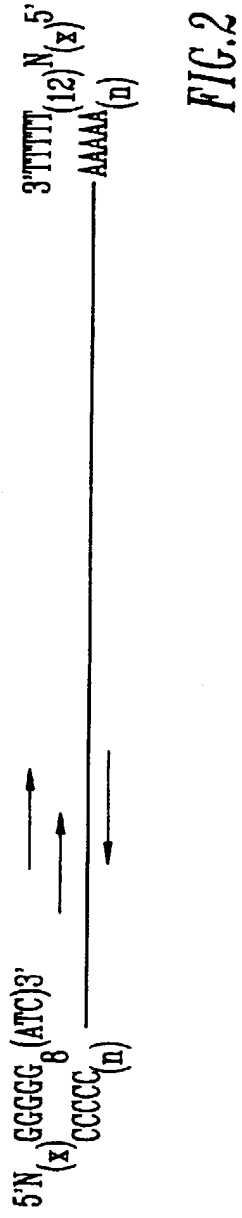
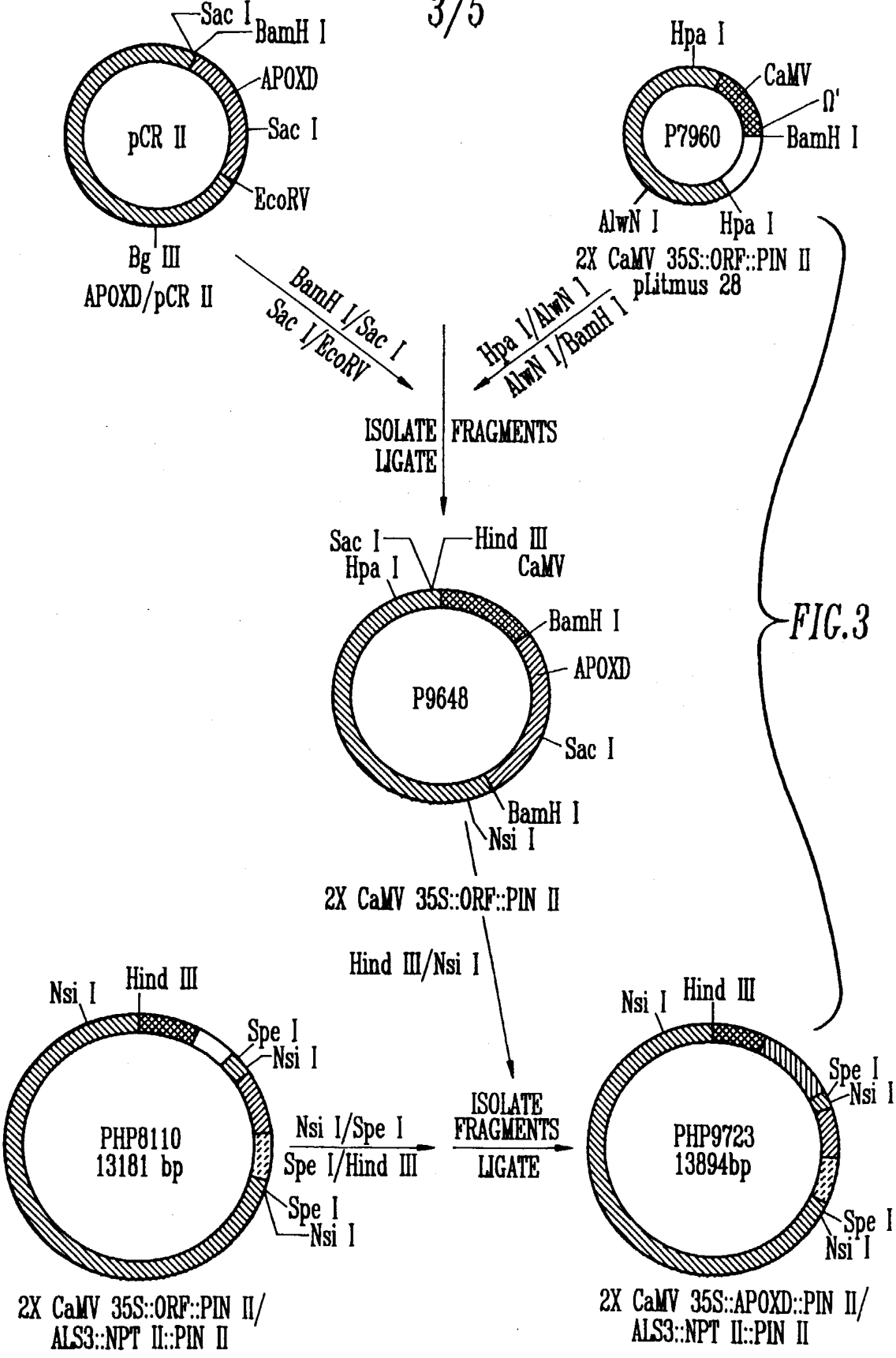


FIG.2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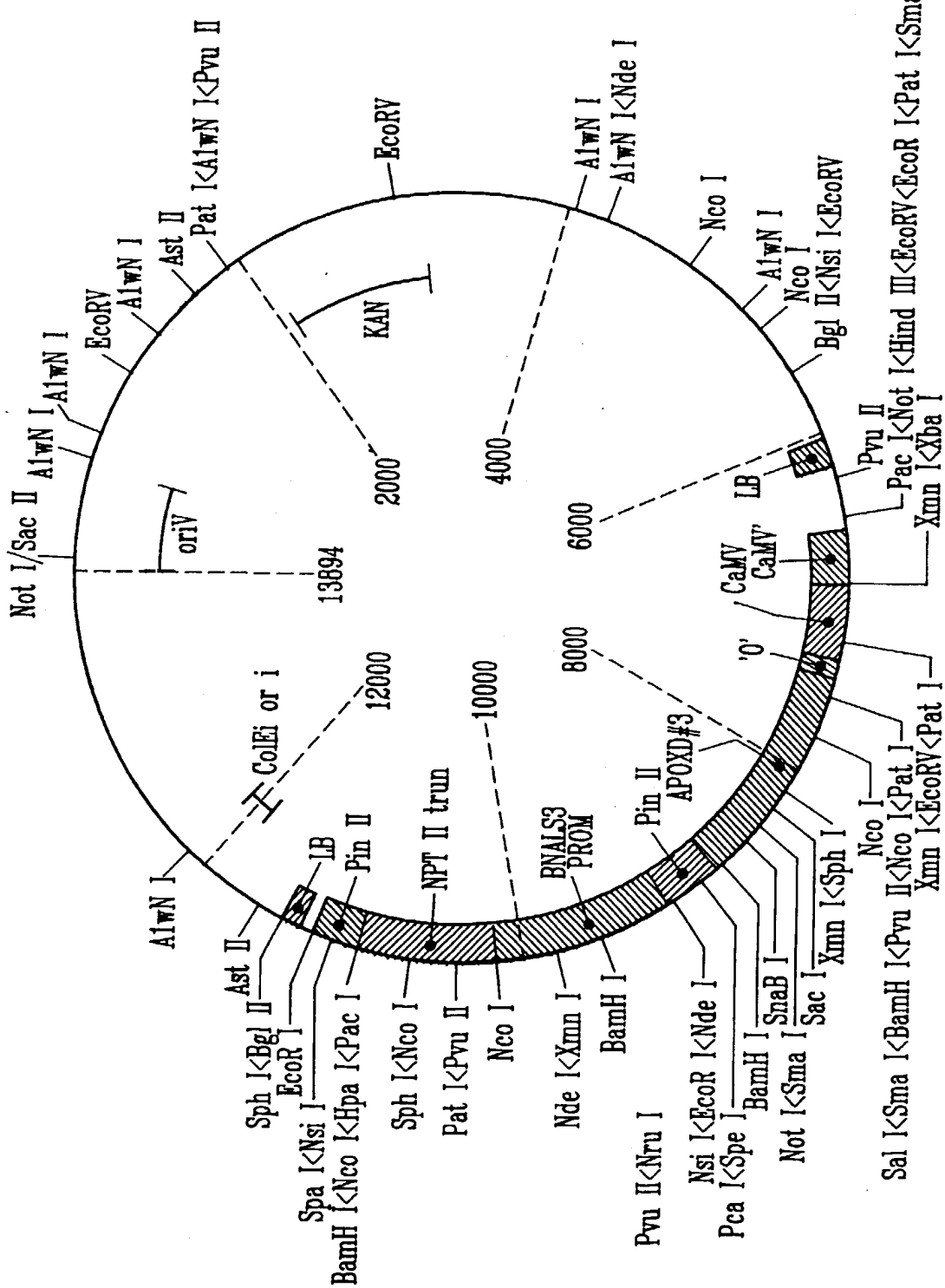
BamH I
APOXD
Sac I
EcoRV
pCR II
Bg III
APOXD/pCR II
BamH I/Sac I
Sac I/EcoRV
Hpa I
CaMV
Ω'
BamH I
P7960
AlwN I
Hpa I
2X CaMV 35S::ORF::PIN II
pLitmus 28
Hpa I/AlwN I
AlwN I/BamH I
ISOLATE FRAGMENTS
LIGATE
Sac I
Hind III
Hpa I
CaMV
BamH I
APOXD
P9648
Sac I
BamH I
Nsi I
2X CaMV 35S::ORF::PIN II
Hind III/Nsi I
Nsi I
Hind III
Spe I
Nsi I
PHP8110
13181 bp
Nsi I/Spe I
Spe I/Hind III
Spe I
Nsi I
ISOLATE
FRAGMENTS
LIGATE
Nsi I
Hind III
Spe I
Nsi I
PHP9723
13894bp
Spe I
Nsi I
2X CaMV 35S::ORF::PIN II/
ALS3::NPT II::PIN II
2X CaMV 35S::APOXD::PIN II/
ALS3::NPT II::PIN II

FIG.3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A1wN I
EcoRV
A1wN I
Ast II
Pat I<A1wN I<Pvu II
EcoRV
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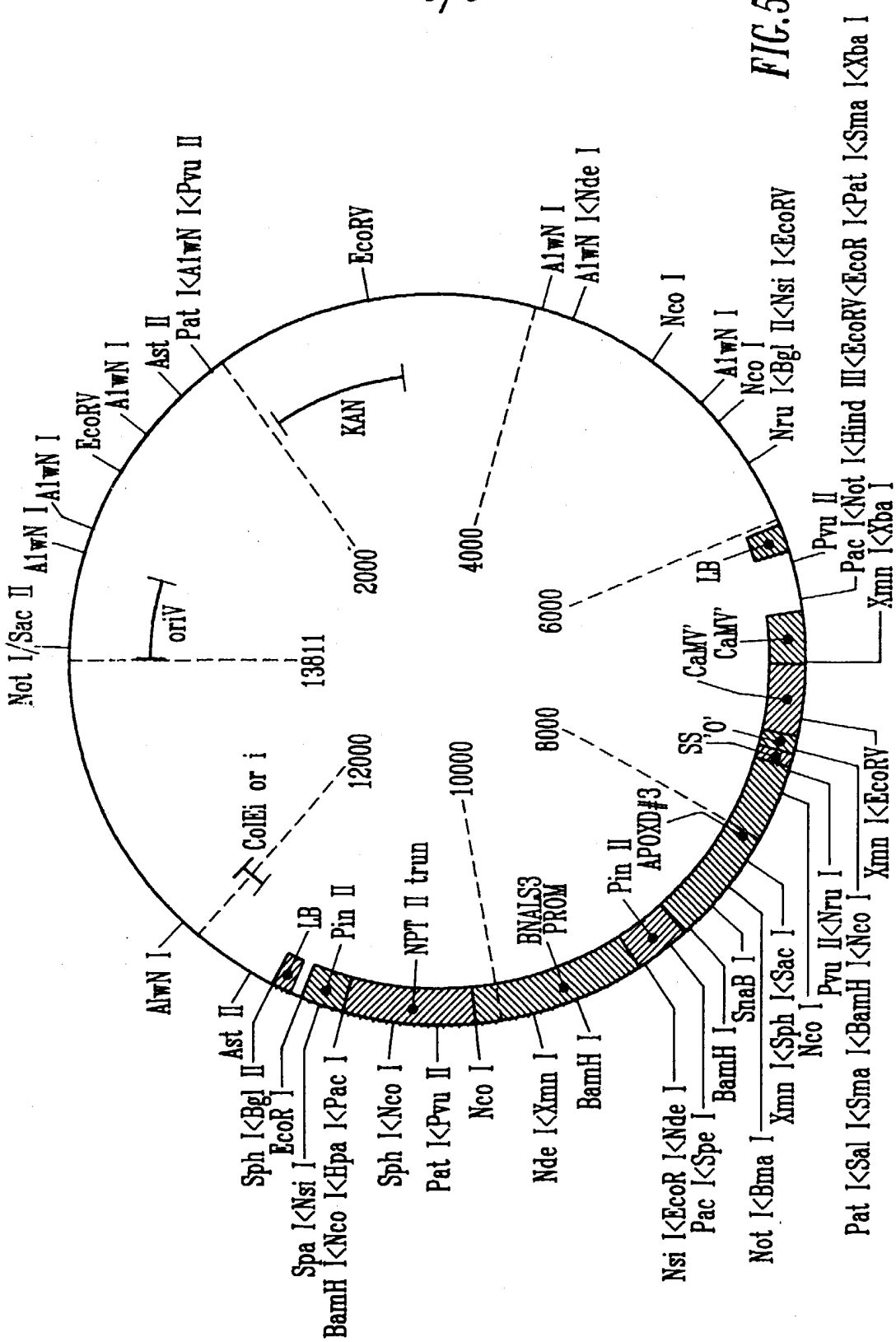
A1wN I<Nde I
Nco I
A1wN I
Nco I
Bgl II<Nsi I<EcoRV
Pvu II
Pac I<Not I<Hind III<EcoRV<EcoR I<Pat I<Sma I<Xba I
Xmn I<Xba I
LB
CaMV
CaMV'
'O'
Nco I
Xmn I<EcoRV<Pat I
Xmn I<Sph I
Sac I
Not I<Sma I
SnaB I
BamH I
Nsi I<EcoR I<Nde I
Pca I<Spe I
Pvu II<Nru I
Pin II
APOXD#3
BNALS3 PROM
BamH I
Nde I<Xmn I
Nco I
Pat I<Pvu II
Sph I<Nco I
NPT II trun
Pin II
LB
Spa I<Nsi I
BamH I<Nco I<Hpa I<Pac I
EcoR I
Sph I<Bgl II
Ast II
A1wN I
ColEi or i
oriV
KAN
Sal I<Sma I<BamH I<Pvu II<Nco I<Pat I
2000
4000
6000
8000
10000
12000
13894

FIG.4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Pat I<AlwN I<Pvu II
EcoRV
AlwN I
AlwN I<Nde I
Nco I
AlwN I
Nco I
Nru I<Bgl II<Nsi I<EcoRV
Pvu II
Pac I<Not I<Hind III<EcoRV<EcoR I<Pat I<Sma I<Xba I
Xmn I<Xba I
CaMV'
CaMV
LB
SS
'O'
Xmn I<EcoRV
Pvu II<Nru I
Xmn I<Sph I<Sac I
SnaB I
BamH I
Not I<Bma I
Nco I
Pat I<Sal I<Sma I<BamH I<Nco I
Nsi I<EcoR I<Nde I
Pac I<Spe I
APOXD#3
Pin II
BamH I
Nde I<Xmn I
BNALS3 PROM
Nco I
Pat I<Pvu II
NPT II trun
Sph I<Nco I
Pin II
Pac I
Spa I<Nsi I
EcoR I
Sph I<Bgl II
Ast II
BamH I<Nco I<Hpa I
LB
ColEi or i
AlwN I
oriV
13811
2000
4000
6000
8000
10000
12000
KAN

FIG.5